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BOARD OF PATENT APPEALS AND INTERFERENCES

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Examiner: L.E. Crane
TOR, et al.	Group: 1600
Serial No.: 08/648,270	
Filed: May 15, 1996	
For: SUBSTITUTED PHENANTHROLINES	

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231 on November 21, 2001

Signed: Mary McFarland
Mary McFarland

APPELLANT'S APPEAL BRIEF

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APPELLANTS' APPEAL BRIEF

This appeal brief, filed in triplicate in connection with the above-captioned patent application, is in response to the final Office Action mailed January 26, 2001, and the Advisory Action mailed April 10, 2001. This appeal is taken pursuant to Rule 1.192, following the Notice of Appeal filed April 23, 2000, along with the requisite fee set forth in 37 CFR § 1.17(c). It is also accompanied by a petition for a five month extension of time and the requisite fee set forth in 37 CFR § 1.17(a).

Appellant also requests an oral hearing under 37 CFR §1.194 and encloses the required fee pursuant to 37 CFR §1.17(d).

The Commissioner is hereby authorized to charge any additional fees which may be required, including extension fees, or credit any overpayment to Deposit Account No. 06-1300, Order No. A-63463-1RFT/RMS/RMK.

I. REAL PARTY IN INTEREST

The real party in interest is The Regents of the University of California, Oakland, California, the owner by assignment of the above-identified patent application.

II. RELATED APPEALS AND INTERFERENCES

Appellants are not aware of related appeals or interferences which will directly affect, be directly affected by, or have a bearing on the Board's decision in this appeal.

III. STATUS OF CLAIMS

The present application was a originally filed with Claims 1-10. Claim 1 was amended and claims 11-19 were added in a Preliminary Amendment dated April 25, 1997. In response to a Restriction Requirement dated August 13, 1997, claims 4-5, 9-10, 12-13 and 17-19 were elected and claims 1-3, 6-8, 11 and 14-16 were cancelled as drawn to non-elected inventions. Claims 4, 5, 10, 12, and 18 were amended and claims 20-24 were added in response to an Office Action dated October 1, 1997. Claims 4-5, 9-10, 12-13 and 17-25 were cancelled and claims 26-43 were added in response to a final Office Action dated June 17, 1998. Claims 26, and 28-43 were amended and claims 44-49 were added in response to a final Office Action dated January 19, 1999. These amendments were not entered (see Advisory Action dated August 2, 1999, Paper No. 21). In a Continued Prosecution Application, filed September 20, 1999, the amendments to Claims 26 and 28-43 were reiterated, Claim 27 was amended, and new claims 44-47 were added. Claims 27-43 were cancelled, Claims 44-45 were amended and new claims 48-49 were added in response to an Office Action dated October 18, 1999. Claims 44-46, and 48 were amended in a Continued Prosecution Application filed January 5, 2001. Claim 48 was amended in response to an Office Action dated January 26, 2001. The claims on appeal, Claims 44-49, as currently pending are set forth in Appendix A.

IV. STATUS OF AMENDMENTS

Subsequent to the final Office Action mailed January 26, 2001, an Amendment and Response was filed, amending Claim 48. An Advisory Action was mailed April 10, 2001, in which the Examiner stated that the amendments would be entered upon filing of a Notice of

Appeal and an Appeal Brief. The claims as set forth in Appendix A incorporate all amendments to date.

V. SUMMARY OF THE INVENTION

The present invention is directed to 1,10-phenanthroline derivatives functionalized at the 3 and/or 8 positions. This is accomplished via the halogenation of 1,10-phenanthroline at the 3 and/or 8 positions. Once halognenated derivatives of 1,10-phenanthrolines are available, additional molecules can be added, such as transition metals, aromatic acetylenes, nucleic acids, nucelotides, nucleosides, proteins, etc.

Claims 44-46 are directed to aromatic derivatives of phenanthroline comprising biological moieties such as nucleic acids (Claim 46), nucleotides (Claim 45) and nucleosides (Claim 44) which may be optionally substituted with transition metals.

In an additional aspect, Claims 47 and 48 are directed to aromatic derivatives of phenanthroline comprising nucleic acid analogs (Claim 47) such as those disclosed in the specification at page 11, lines, 11-29 and, more specifically, the phosphoramidite form of a nucleotide (Claim 48).

In a final aspect, Claim 49 is directed to the specific transition metals which may be incorporated into the phenanthroline derivatives disclosed in Claims 44-48.

VI. ISSUES

The final Office Action mailed on January 26, 2001, presents two issues for which Appellants request review (listed only as claims still pending):

1) An issue of patentability under the enablement requirement of 35 U.S.C. § 112, first paragraph with regard to Claims 44-49; and,

2) An issue of patentability under the written description requirement of 35 U.S.C. § 112, first paragraph with regard to Claims 44-49.

VII. GROUPING OF CLAIMS

For purposes of this appeal, Claims 44-47, and Claim 49 (to the extent that Claim 49 depends on Claims 44-47) are grouped together and stand or fall together independent from the others.

The second group comprises Claim 48 and Claim 49 (to the extent that Claim 49 depends on Claim 48) are grouped together and stand or fall together independent from the others.

VIII. ARGUMENT

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH, SHOULD BE WITHDRAWN.

1. ARGUMENT IN SUMMARY

According to the final rejection mailed January 26, 2001, the specification is entirely prospective. Specifically, the Examiner argues that the specification lacks specific embodiments which show the applicant had possession of the compounds indicated, that a substantial number

of compounds had been made, and that there is a complete absence of requisite guidance to permit the ordinary practitioner to make the compounds without undue experimentation. The Appellants submit that a) the specification provides ample guidance to one of skill in the art to make and use the compounds of the invention; and, b) the specification provides specific examples of making at least two aromatic acetylene derivatives of phenanthroline.

2. DETAILED ARGUMENTS

a. The final rejection of Claims 44-49 under 35 U.S.C. § 112, first paragraph as allegedly not being supported by an enabling disclosure is improper and should be withdrawn

In rejecting Claims 44-49 under 35 U.S.C. under § 112, first paragraph, for lack of enablement, the Examiner's has reiterated his position that the specification is entirely prospective. Specifically, the Examiner argues that the specification lacks specific embodiments which show the applicant had possession of the compounds indicated, that a substantial number of compounds had been made, and that there is a complete absence of requisite guidance to permit the ordinary practitioner to make the compounds without undue experimentation.

It is well settled law that the specification must enable the scope of the claimed invention, but that the specification need not provide a specific description for each and every embodiment covered by the claimed invention. *See, e.g., Vas-Cath Inc. v. Mahurkar*, 19 USPQ2d 1111, 1115 (Fed. Cir. 1991). That the claimed invention covers a wide variety of compounds amenable to synthesis in accordance with the claimed invention is not relevant to the issue of enablement. Rather, the test of enablement requires that the specification, in light of the prior art, provides

ample guidance to one of skill in the art, to make and use the compounds of the invention. Appellants maintain that the specification provides specific examples of making at least two aromatic acetylene derivatives of phenanthroline. This alone is sufficient teaching to enable the scope of independent claims 44-46 and 48.

i. Synthesis of Aromatic Acetylene Derivatives of Phenanthrolines

The present invention is directed to the synthesis of 1,10-phenanthroline derivatives functionalized at the 3 and /or 8 positions. This is accomplished by the halogenation of 1,10-phenanthroline at the 3 and/or 8 positions. Once halogenated derivatives of 1,10-phenanthrolines are available, additional molecules can be added, such as transition metals, aromatic acetylenes, nucleic acids, nucleotides, nucleosides, proteins, etc.

The synthesis of aromatic acetylene derivatives of phenanthrolines requires: a) halogenation, particularly bromination, of 1,10-phenanthroline at the 3- and/or 8- position; b) optional addition of a transition metal to the halogenated 1,10-phenanthroline to form transition metal derivatives of 1,10 phenanthroline; and, c) palladium cross coupling to covalently attach acetylated aromatic compounds such as nucleic acids, nucleotides and nucleosides to the halogenated 1,10 phenanthroline (which may comprise transition metals).

Halogenation of 1,10-phenanthroline begins with commercially available 1,10-phenanthroline monohydrochloride monohydrate. A typical procedure using bromine is provided in working example 1. As outlined at page 23, lines 5-25:

> In a typical procedure, a solution of the 1,10-phenanthroline monohydrochloride monohydrate(10 g, 43 mmol) in nitrobenzene (20 ml) was heated to 130-140 °C in a 250 ml 3-neck flask. Bromine (3.3 l, 64 mmol in 9.3 ml nitrobenzene) was

added dropwise over a period of 1 hr. Upon the addition of bromine, the 1,10-phenanthroline went into solution. After stirring for 3 hr at the same temperature, the reaction mixture was cooled to room temperature, treated with concentrated ammonium hydroxide (100 ml) and extracted with dichloromethane (3X50 ml). The combined organic layers were washed with water (3X50 ml) and dried ($MgSO_4$). Concentration in vacuum afforded a suspension of the products in nitrobenzene. The nitrobenzene was removed by dissolving the suspension in dichloromethane (10 ml) and filtering it through silica gel (300 ml) using dichloromethane as the eluent. After the nitrobenzene eluted out, the products were recovered by gradually increasing the polarity of the eluent up to 10% MeOH in CH_2Cl_2. Flash column chromatography (0.6% MeOH in CH_2Cl_2) afforded 3-bromo-phenanthroline (3.6 g, 33% yield, m.p. 164-167°C) and the 3,8-bromo-phenanthroline (2.4 g, 17% yield, m.p. 270-273°C) as white powders. Higher solvent polarity (10% MeOH in CH_2Cl_2) elutes unreacted 1,10-phenanthroline (ca. 4 g) that can be recycled.

A typical reaction for adding transition metals to halogenated 1,10-phenanthrolines is described at page 27, lines 4-13:

> In a typical reaction, the ligand **3a** (0.1 g, 0.26 mmol) in degassed DMF (10 ml) was treated under argon with a solution of K_2RuCl_5 (33 mg, 0.08 mmol) in water (4 ml) containing 1 drop of 6N HCl. The solution was refluxed for 1 h. Sodium hypophosphite (38 mg, 0.44 mmol) in water (1 ml) was added, and reflux was continued for 1 h. After cooling to 60°C, the reaction mixture was treated with potassium hexafluorophosphate (48 mg, 0.26 mmol) as a 10% aqueous solution, cooled to RT and concentrated *in vacuo*. Silica-gel chromatography using 1% aqueous 0.5 M KNO_3 in acetonitrile as eluent afforded Ru(**3a**)$_3$. ^{1}H NMR (CD_3CN) d 8.75 (d, *J*=1.3 Hz, 2H, H2,9), 8.27 (s, 2H, H5,6), 8.18 (d, *J*=1.3 Hz, 2H, H4,7), 7.45 (m, 10H, phenyl).

Chemical compositions of ligand **3a** and Ru(**3a**)$_3$ are found in Tables 1 and 2 of the specification respectively.

With respect to the palladium-catalyzed coupling reactions, transition metal catalyzed reactions have played a role in organic synthesis for a long time. Over twenty five years ago the use of palladium as a catalyst for coupling reactions was disclosed. Since then, the scope of

palladium-catalyzed reactions has been expanded and used to join a wide variety of chemical reactants via the formation of a carbon-carbon bond. See for example, U.S. Patent No. 6,136,157; a copy of which is attached as Exhibit A, which describes the efforts devoted to extending the scope of palladium-catalyzed reactions.

Briefly, palladium-catalyzed cross coupling reactions are used to form carbon-carbon bonds between halogenated carbons and other carbon atoms, such as alkynes, alkenes and aromatic acetylenes. This reaction is quite versatile and widely used to synthesize a large number of compounds. In particular, palladium-catalyzed cross coupling reactions have been used to couple nucleosides to a variety of compounds. For example, Robins and Barr report details of a high-yield palladium-catalyzed coupling procedure which provides direct access to 5-alkynyluracil bases and nucleosides from terminal alkynes and readily available 5-iodouracil derivatives. See Robins and Barr, (1983) *J. Org. Chem.*, 48:1854-1862, a copy of which is attached as Exhibit B. Moriarty et al. describe the use of a palladium catalyzed coupling reaction between 8-iodo derivatives of O-TBDMS protected adenosine, 2'-deoxyadenosine, and 2',3'-dedeoxyadenosine to obtain 8-substituted nucleosides. Moriarty, et al., (1990) *Tetrahedron Letters*, 41:5877-5880, a copy of which is attached as Exhibit C. Thus, the use of palladium catalyzed-cross coupling reactions in the coupling of nucleosides with other compounds is well known in the art.

The present invention utilizes a palladium-catalyzed cross coupling reaction to make 1,10-phenanthrolines derivatives. Guidance for palladium-catalyzed cross coupling reactions between an aromatic acetylene and halogenated 1,10-phenanthroline is depicted in Scheme II, page 18 of the specification. Other palladium-catalyzed reactions include: a) reacting an

halogenated 1,10-phenanthroline with acetylene, to form a 3- or 3,8-acetylene-phenanthroline and then coupling to an halogenated aromatic group (Scheme III, page 18 of the specification); b) coupling transition metal complexed 1,10-phenanthrolines with an aromatic acetylene (Scheme IV, page 20); c) coupling a transition metal complexed 3-acetylene phenanthroline with an aromatic bromine (Scheme V, page 20); and d) coupling an optionally transition metal complexed 1,10-phenanthroline with a halogenated nucleosides (page 20, line 8 through page 21, line 2).

Moreover, specific reaction conditions are disclosed in example 3, page 29, lines 17-24:

> A representative procedure for the palladium-mediated cross-coupling reactions between **4** and aromatic acetylenes is as follows. A mixture of **4** (50 mg, 0.052 mmol), $(Ph_3P)_2PdCl_2$ (4 mg, 0.0057 mmol) and CuI (0.5 mg, 0.0026 mmol) was treated with a degassed solution of 4-ethynyltoluene (11 µl, 0.11 mmol) in DMF (5 ml) and triethylamine (3 ml) for 1 hour at room temperature under Argon. The crude reaction mixture was evaporated to dryness and the product **6** was obtained in 91% yield as an orange-red powder after successive crystallizations from dichloromethane-ethanol.

The chemical structures for compounds **4** and **6** are found on pages 31 and 32 respectively.

The substrates required for the palladium cross coupling reactions described in the present invention are widely available. Aromatic acetylenes can be made using techniques well known in the art or obtained commercially. See specification at page 20, lines 3-7. Halogenated bases and nucleosides are commercially available. *See* Robbins and Barr, Exhibit C. Moreover, halogenated bases and nucleosides can be synthesized using terminal alkynes and halogenated nucleosides as starting materials. *See* Moriarty et al., Exhibit B.

In further support of the enablement of the specification, appellants include a recent article which describes the synthesis of metal containing nucleosides. See Hurley and Tor

(1998), *J. Am. Chem. Soc.*, 120:2194-2195, enclosed herein as Exhibit D. The appellants are not using subsequent work to supplement the disclosure of the application; rather, the subsequent work is presented to show that the utility asserted and shown in the application is supported by further research, and that the specification fully enables the synthesis of 1,10-phenanthroline derivatives substituted at the 3- and/8-positions. See *In re Wilson*, 135 USPQ 442, 444 (CCPA 1962); *Ex parte Obukowicz*, 27 USPQ 2d 1063 (BPAI 1993); *Gould v. Quigg*, 3 USPQ 2d 1302,1305 (Fed. Cir. 1987):

> "it is true that a later dated publication cannot supplement an insufficient disclosure in a prior dated application to render it enabling. In this case the later dated publication was not offered as evidence for this purpose. Rather, it was offered . . . as evidence that the disclosed device would have been operative."

Hurley and Tor report the synthesis of novel Ru^{II}- and Os^{II}-containing nucleosides and their phosphoramidite derivatives. The methods used to synthesis these metal-modified nucleosides are the same as those disclosed in the present invention and outlined above: a) halogenation of 1,10-phenanthrolines; b) the optional incorporation of transition metals to form transition methal complexed 1,10-phenanthroline derivatives, and, c) palladium-catalyzed cross coupling reactions between acetylated nucleosides and transition metal complexed 1,10-phenanthrolines. See Exhibit D, page 2194. In addition, the Hurley and Tor reference utilize solid phase phosphoramidite chemistry to synthesize the corresponding metal-modified phosphoramidite. Exhibit D, page 2194; the specification, page 21, lines 1-2.

In further support of the proposition that the specification is enabling, the appellants submit the declaration of Dr. Tom Meade. Dr. Meade's declaration is enclosed as Exhibit E.

In paragraph 7, Dr. Meade states that the bromination procedure used to make

functionalized derivatives of 1,10 phenanthroline, is sufficiently described to allow one of skill in the art to make these compounds without undue experimentation.

Likewise, in paragraph 8, Dr. Meade states that the reaction described for adding the transition metal ruthenium to functionalized derivatives of 1,10 phenanthroline may be adapted for the addition of other metal ions without undue experimentation.

In paragraphs 9 and 10, Dr. Meade is of the opinion that sufficient guidance for using the palladium catalyzed cross coupling reaction to couple nucleosides to aromatic acetylene derivatives of 1,10 phenanthroline is provided in the specification.

In paragraph 11, Dr. Meade states that methods for synthesizing oligonucleotides are well known in the art. Moreover, Dr. Meade is of the opinion that the reference in the specification to the use of solid-phase phosphoramidite chemistry for the synthesis of the compounds of the present invention is sufficient to allow a person of skill in the art to make these compounds without undue experimentation.

Appellants recognize that the declaration of Dr. Meade is opinion evidence. However, appellants submit that Dr. Meade's testimony is entitled to some consideration and weight as a person skilled in the art as it provides a factual assessment of whether the specification is enabling. See M.P.E.P § 716.01(c). Thus, the Examiner's statement that the "opinion and belief of a third party has been deemed to be nothing more than self serving" clearly does not comport with the guidelines outlined in M.P.E.P § 716 and 716.01(c). See the Advisory Action, dated April 10, 2001.

Accordingly, in light of the prior art and teachings in the specification, appellants submit that the skilled artisan would find guidance for making the compounds described in the

specification. Accordingly, appellants submit that Claims 44-49 are enabled and request withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

b. The final rejection of Claims 44-49 under 35 U.S.C. § 112, second paragraph as allegedly not be supported by a written disclosure is improper and should be withdrawn

In rejecting Claims 44-49 under 35 U.S.C. under § 112, first paragraph, for lack of written description, the Examiner's has reiterated his position that the specification is broadly descriptive of the claimed invention, but lacks examples as how to incorporate the claimed compounds into an oligonucleotide.

To satisfy the written description requirement, an applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention as now claimed. The test for sufficiency of support is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." *See, e.g., Ralston Purina Co. v. Far-Mar-Co., Inc.*, 772 F.2d 1570,1575 (Fed. Cir. 1985) (quoting *In re Kaslow*, 707 F.2d 1366, 1375 (Fed, Cir. 1983)).

An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565,1572 (Fed. Cir. 1997). Possession may be shown in a variety of ways including description of an actual reduction to practice, or by showing that the invention was "ready for patenting" such as by the disclosure of drawings or structural chemical formulas

that show that the invention was complete. In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus. *See e.g., The Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 1568 (Fed. Cir. 1997).

As stated above, the specification provides working examples for the bromination of 1, 10-phenanthroline (see Example 1 at page 23, lines 5-25); for the addition of transition metals to halogenated 1, 10-phenanthroline (see page 27, lines 4-13); for the palladium-catalyzed cross coupling reactions (see page 20-22, Schemes II-V). Moreover, specific reaction conditions are disclosed in Example 3, page 29, lines 17-24. Finally, chemical structures for the compounds are found on pages 31 and 32.

Appellants have provided additional supporting evidence that the specification provides a written description as required by 35 U.S.C. § 112, first paragraph. For example, appellants have provided Exhibit D, a paper subsequently published by the inventor that describes the synthesis of metal containing nucleosides using the methods disclosed in the present invention and outlined above. As the methods used in the publication are those disclosed in the specification, this paper supports the appellants position that the inventor was in possession of the invention at the time of filing.

In addition, appellants have provided the declaration of Dr. Tom Meade, enclosed as Exhibit E. As evidenced by Dr. Meade's curriculum vitae, Dr. Meade has worked and published in the area of the invention. Dr. Meade in paragraphs 7-11 states that the reactions for making

functionalized derivatives of 1, 10-phenanthroline, for adding transition metals, and the use of palladium catalyzed cross coupling are sufficiently described to allow one of skill in the art to make the compounds of the invention. Moreover, Dr. Meade states that as the methods for synthesizing oligonucleotides were well known in the art, that he is of the opinion that the reference in the specification to the use of solid-phase phosphoramidite chemistry for the synthesis of the compounds of the present invention is sufficient to allow a person of skill in the art to make these compounds.

Appellants submit that Dr. Meade's testimony is entitled to some consideration and weight as a person skilled in the art as it provides a factual assessment of whether the written description is sufficient to convey the invention as presently claimed. See M.P.E.P § 716.01(c). Thus, the Examiner's statement that the "opinion and belief of a third party has been deemed to be nothing more than self serving" clearly does not comport with the guidelines outlined in M.P.E.P § 716 and 716.01(c). See the Advisory Action, dated April 10, 2001.

Applicants submit that the synthetic schemes, accompanying descriptions and examples, convey with reasonable clarity to those skilled in the art that, as of the filing date sought, the inventor was in possession of the invention. Accordingly, appellants submit that Claims 44-49 satisfy the written description requirement and request withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

c. Conclusion

In conclusion and in light of the above, Appellants believe that the above arguments warrant reconsideration and withdrawal of the outstanding final rejections of claims 44-49 under 35 U.S.C. § 112, first and second paragraph.

Dated: 11/21/01

Respectfully submitted,

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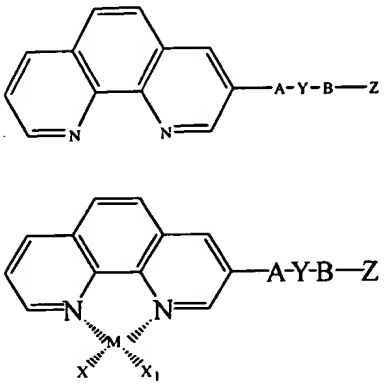
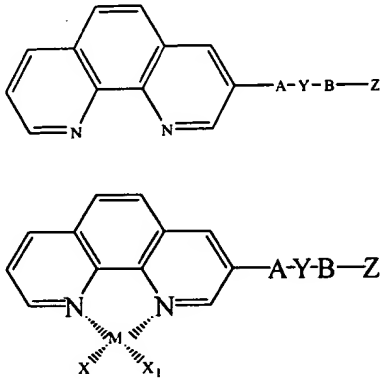
1065616.RMK

44. (Twice Amended) A compound represented by one of the formulae:



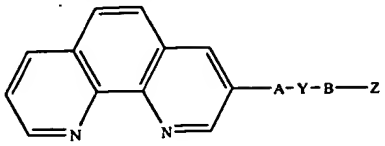
wherein
M is a transition metal ion;
the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-, -N=N-, and -CH=N-;
X and X_1 are co-ligands and wherein at least one of X and X_1 is present; and
Z is a nucleosidyl moiety attached via the base.

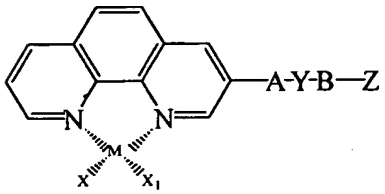
45. (Twice Amended) A compound represented by one of the formulae:



wherein
M is a transition metal ion;
the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-, -N=N-, and -CH=N-;
X and X_1 are co-ligands and wherein at least one of X and X_1 is present; and
Z is a nucleotidyl moiety attached via the base.

46. (Twice Amended) A compound represented by one of the formulae:

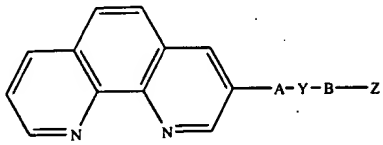


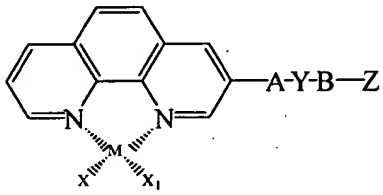


wherein
M is a transition metal ion;
the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-, -N=N-, and -CH=N-;
X and X_1 are co-ligands and wherein at least one of X and X_1 is present; and,
Z is a nucleic acid moiety attached via a base.

47. A compound according to claim 46, wherein said nucleic acid moiety comprises a nucleic acid analog.

48. (Thrice Amended) A compound represented by one of the formulae:





wherein
M is a transition metal ion;
the A-Y-B moiety is selected from the group consisting of -C≡C-, -CH=CH-, -N=N-, and -CH=N-;
X and X_1 are co-ligands and wherein at least one of X and X_1 is present; and,
Z is a phosphoramidite nucleotidyl moiety attached via the base.

49. A compound according to claims 44, 45, 46 or 48 wherein M is selected from the group consisting of ruthenium, rhenium and osmium.

Appendix B

US006136157A

United States Patent [19]

Lindeberg et al.

[11] Patent Number: 6,136,157

[45] Date of Patent: Oct. 24, 2000

[54] METHOD FOR ORGANIC REACTIONS

[75] Inventors: Gunnar Lindeberg; Mats Larhed; Anders Hallberg, all of Uppsala, Sweden

[73] Assignee: Labwell AB, Uppsala, Sweden

[21] Appl. No.: 09/180,673

[22] PCT Filed: May 14, 1997

[86] PCT No.: PCT/SE97/00794

§ 371 Date: Nov. 24, 1998

§ 102(e) Date: Nov. 24, 1998

[87] PCT Pub. No.: WO97/43230

PCT Pub. Date: Nov. 20, 1997

[30] Foreign Application Priority Data

May 14, 1996 [SE] Sweden 9601856
Oct. 25, 1996 [SE] Sweden 9603913

[51] Int. Cl.[7] C07F 1/00

[52] U.S. Cl. 204/157.6; 204/157.69

[58] Field of Search 204/157.6, 157.69

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(List continued on next page.)

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[57] ABSTRACT

Organic reactions catalyzed by palladium, except Pd/C, are conducted with heating by microwave energy. The preferred inorganic reactions involved are coupling reactions in which a new carbon-carbon bond is formed. Preferred reactions are the Heck, Stille and Suzuki reaction. The method provides high yields in very short reaction times.

22 Claims, No Drawings

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METHOD FOR ORGANIC REACTIONS

This is a national stage application of PCT/SE97/00794 filed May 14, 1997.

FIELD OF THE INVENTION

The present invention relates to a method for transition metal catalyzed organic reactions comprising a heating step. More precisely, the invention relates to organic reactions catalyzed with palladium, except Pd/C, under microwave energy. One type of organic reactions concerned are coupling reactions, in which new carbon-carbon bonds are formed.

BACKGROUND OF THE INVENTION

Transition metal catalyzed reactions have played a role in organic synthesis for a very long time. Twenty five years ago the first examples of the use of palladium as a catalyst for coupling reactions were disclosed. Since then enormous efforts have been devoted to extend the scope of palladium-catalyzed reactions. Among these, the Heck reaction[1], the Stille reaction[2] and the Suzuki reaction[3] probably represent the most applied and most reliable reactions considering the reactions delivering new carbon-carbon bond formation. These reactions allow the presence of a wide variety of substituents attached to the reactants and merit special attention due to the simplicity of the experimental procedures. The Heck reaction, which is a vinylic substitution reaction, is most frequently conducted with olefines and organo halides or triflates as reactants. Couplings of organotin reagents with organo halides or triflates are named Stille reactions. The related couplings of organoboronic acids and/or organoboronic esters with organo halides or triflates are named Suzuki reactions. These reactions are of utmost importance in organic synthesis and have all found use in Combinatorial Chemistry (CC), sometimes as key reactions in the creation of chemical libraries[4]. Combinatorial Chemistry is conducted either in solution or preferably on solid phase. Combinatorial Chemistry combined with High Throughput Screening (HTS) has revolutionized the Drug Discovery Programmes in the pharmaceutical industry during the last few years[5].

In Combinatorial Chemistry the reaction time factor is of importance. Rapid reactions are desired. The long reaction times often required in the palladium-catalyzed coupling reactions presented above is therefore in this respect a severe limitation. While some combinations of reactants allow fast conversions, and efficient use in Combinatorial Chemistry have been demonstrated, the majority of reactant combinations require unacceptable reaction times for completion. With regard to the palladium-catalyzed Heck, Stille or Suzuki reactions, attempted enhancement of the conversion rate by increasing the reaction temperature to over 130–150° C. most often leads to collapse of the catalytic system before a full conversion is achieved. Product decomposition is frequently observed and a mixture of undesired side products are formed.

PRIOR ART

Microwave assisted transition-metal catalyzed reactions of double bonds, including hydrogenation, catalyzed by RaNi or Pd/C,[6] and addition of chloronated hydrocarbons to double bonds, catalyzed by CuI[7] have been reported.

SUMMARY OF THE INVENTION

The present invention provides a method for palladium, except Pd/C, catalyzed organic reactions comprising a heating step performed with microwave energy.

In a preferred embodiment, the reactions are coupling reactions such as the Heck, Stille and Suzuki reactions.

Such reactions can be depicted as follows:

i) $Org^1X+H\text{-olefin} \rightarrow Org^1\text{-olefin}$

ii) $Org^1X+R^1{}_3Sn\text{-}Org^2 \rightarrow Org^1\text{-}Org^2$

iii) $Org^1X+R^2{}_2B\text{-}Org^3 \rightarrow Org^1\text{-}Org^3$

in which Org is an organic compound and Org^1 is aryl, heteroaryl, vinyl, acetylenyl, alkyl, allyl, benzyl, acyl, benzoyl, or mono or poly substituted aryl, heteroaryl, vinyl, acetylenyl, alkyl, allyl, benzyl, or benzoyl;

X is a halide, triflate, mesitylate, nonaflate, carbonylhalide, sulfonylhalide, perfluoroalkylsulfonate, arylphosphate, alkylphosphate, diarylarsine, diarylphosphine, diarylstibine, aryliodonium or diazonium salt;

the double bond in the H-olefin is unsubstituted or mono, di or tri substituted;

R^1 is alkyl, aryl, heteroaryl or mono or poly substituted alkyl, aryl or heteroaryl;

Org^2=Org^3 and is aryl, heteroaryl, vinyl, acetylenyl, alkyl, allyl, benzyl or mono or poly substituted aryl, heteroaryl, vinyl, acetylenyl, alkyl, allyl, benzyl;

R^2 is OH, alkoxy, aryloxy or heteroaryloxy.

The reactions may be performed on a solid support, wherein Org^1, Org^2 or H-olefin are preferably attached to a solid support via a linker.

Furthermore, the present invention provides for use of palladium-catalyzed coupling reactions in combinatorial chemistry and for creation of chemical libraries.

DETAILED DESCRIPTION OF THE INVENTION

1. PALLADIUM-CATALYZED COUPLINGS IN SOLUTION

Microwave assisted methodology[8] is based on a fast heating of the reaction mixtures to high temperatures. The inventors surprisingly found that the Heck, Stille and Suzuki reactions in which labile organotransition metal intermediates play a pivotal role in the catatalytic cycle, are compatible with the extreme temperatures used in these reactions. It was found that the Heck, Stille and Suzuki reactions, after very short reaction times (2–7 minutes) when applying microwave assisted technology, allowed isolation of reaction products in high yields and also with high purity, see TABLE 1 below. The corresponding reactions performed with standard heating required hours to reach satisfactory yields according to literature, as denoted in TABLE 1. While an increase of the reaction temperature in the latter cases often provided complex reaction mixtures, the microwave assisted reactions were leading to essentially no undesired side products. Preferably the microwave energy is solely or predominantly in the form of a standing wave.

The inventors have reacted aryl iodides, aryl bromides and aryl triflates with various olefins (Heck reactions). These olefins were chosen to permit comparison of microwave-assisted reactions with conventionally heated reactions, with respect to regio- and stereoselectivity, as well as double bond isomerization. The reactions were conducted in sealed pyrex vessels and a commercially available reactor producing continuous irradiation was used. Our initial screening experments, with different solvents suggested that DMF was suitable for all reactant combinations tested. Acetonitrile, ethanol, water, DME and NMP are examples of other suitable solvents. With the exception of reaction scale, solvent and heating procedure, the reactions were performed under reaction conditions identical to the original procedures

as described in the references cited, in order to enable an accurate comparison. The inventors intended to utilize microwave assisted reactions in Combinatorial Chemistry and the primary objective was minimization of the reaction times. Irradiation effects were altered to allow full conversions of the arylating agents in less than seven minutes.

Methyl acrylate was smoothly converted in 3.5 min at 60 W, to the corresponding cinnamic acid ester in a standard Heck reaction medium. The same transformation could be conducted at a lower power although a longer reaction time was required. In order to achieve a full consumption of 1-iodonaphthalene in a reaction with acrylonitrile in the same reaction time, a power of 80 W was used. While methyl acrylate provided the E isomer exclusively, the reaction with acrylonitrile produced a mixture of the E and Z isomers (E/Z=4/1) as expected from related standard reactions with this olefin. Arylation of styrene with iodobenzene delivered a terminal/internal ratio of 19/1, in accordance with the original procedure. Microwave assisted arylation of dihydrofuran according to Larock's original procedure furnished a mixture of double bond isomers. 2-Phenyl-2, 3-dihydrofuran was isolated in fair yield. A reaction time of 6 min at 30 W was needed in this case, to be compared with 24 hours at 80° C. when standard heating was employed. For studies of the regioselectivity with regard to the direction of insertion, alkyl vinyl ethers were used as substrates. Procedures have been developed that allow either electronically controlled internal arylation by application of bidentate ligands or alternatively, chelation-controlled terminal arylation. Microwave assisted arylation of butyl vinyl ether with aryl triflate provided a mixture of a-arylated vinyl ether and the corresponding acetophenone. 4-Acetyl tert. butylbenzene could be isolated in good yield after conducting the reaction in the presence of a minor amount of water. The reactions of dimethylaminoethyl vinyl ether with 2-naphthyl triflate as the substrate constitutes an example of a sluggish reaction that requires 9 hours at 60° C. to afford fair yields of coupled product. In the microwave assisted reaction, it was essential to employ a low effect, 35W, to avoid decomposition of the catalytic system. A longer reaction time, 7 min, was therefore needed. A highly regioselective arylation at the terminal carbon occurred, and equal amounts of the E and Z isomers were formed, in full agreement with the reference procedures. Allyldimethylamine and allyltrimethylsilane were both successfully arylated at the internal olefinic carbon with high regioselectivity after 5 minutes.

Suzuki coupling of phenyl boronic acid with 4-bromotoluene as well as Stille coupling of 4-acetyl phenyl triflate occurred smoothly with microwave irradiation.

Arylation of styrene with 4-bromo-1-iodobenzene delivered a terminal/internal ratio of 15/1 in accordance with the original procedure. The result from this reaction shows that the microwave irradiation provides the same selectivity, with respect to halide displacement as compared to the original reaction and substitution of the iodoatom occurs exclusively. No products derived from bromo substitution were formed, demonstrating the potential of microwave irradiation technology for combinatorial chemistry. The bromo atom remains intact and provides a handle for further consecutive palladium-catalyzed transformations.

2. PALLADIUM-CATALYZED COUPLINGS ON SOLID SUPPORT

The inventors have found that palladium-catalyzed reactions on polymer-support were compatible with the microwave assisted methodology. Preferably the microwave energy is solely or predominantly in the form of a standing wave. High yields of products with high purity were isolated after short reaction times. No or very limited decomposition of the solid phase was observed. Examples of microwave-assisted Suzuki couplings on solid phase are demonstrated in TABLE 2 below. We coupled 4-iodo and 4-bromo- benzoic acid to Rink resin[18] (TENTAGEL™ (see footnote 19), a grafted copolymer consisting of a low crosslinked polystyrene matrix on which polyethylene-glycol (PEG or POE) is grafted) and conducted the palladium-catalyzed reactions with aryl and heteroaryl boronic acids substituted with a variety of substituents. Both arylbromo and aryliodo groups on the resin are good coupling partners with organoboronic acids.

One example of a Stille coupling on solid phase and one example of a Heck coupling on solid phase are shown in TABLE 3.

Entry 15 in TABLE 2 demonstrates that the microwave technology permit selective coupling of 4-bromophenylboronic acid to aryl iodide attached to the resin. The organoiodide is more reactive and the bromo atom is not displaced. A high chemoselectivity is achieved and the bromo atom constitutes a handle for consecutive reactions.

TABLE 1

Palladium-Catalyzed Coupling Reactions in Solution under Microwave Irradiation

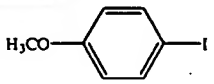
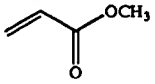
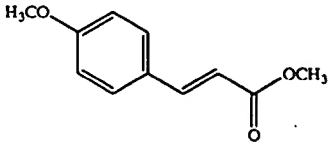
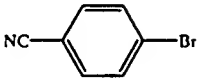
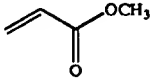
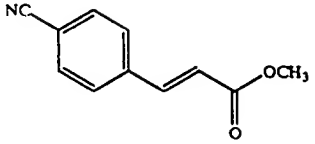
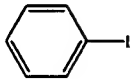
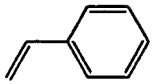
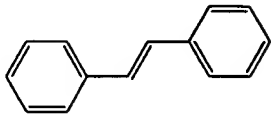
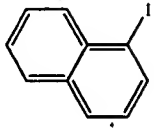
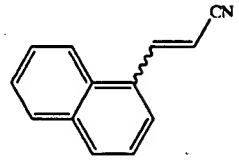
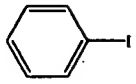
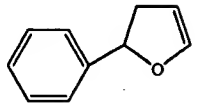
entry	aryl halide or aryl triflate	olefine or organometallic	time effect	product	isolated yield[a]	conventional heating time (min)	conventional heating yield (%)
1			3.50 min. 60 W $Pd(OAc)_2$, DMF		70%	300	68%[9]
2			3.50 min. 60 W $Pd(OAc)_2$, DMF o-$(tol)_3P$		94%	120	70%[10]
3			2.50 min. 90 W $Pd(OAc)_2$, DMF		87%	120	75%[9]
4			3.50 min. 80 W $Pd(OAc)_2$, DMF		90%		
5			6.00 min. 30 W $Pd(OAc)_2$, Ph_3P DMF, Bu_4NCl		58%	1440	76%[11]

TABLE 1-continued

Palladium-Catalyzed Coupling Reactions in Solution under Microwave Irradiation

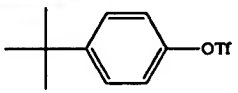
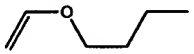
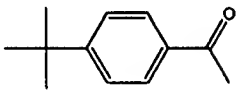
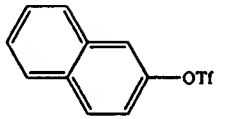
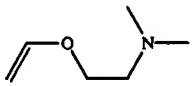
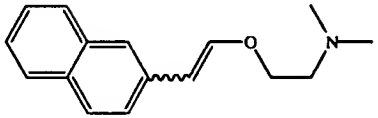
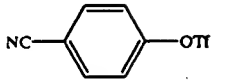
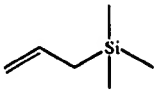
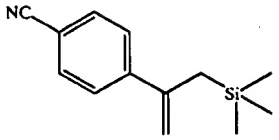
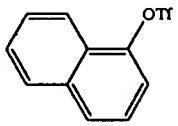
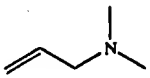
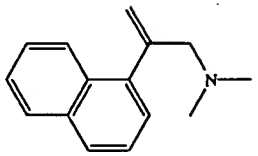
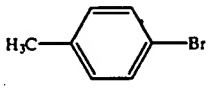
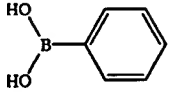
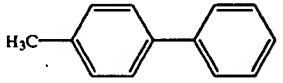
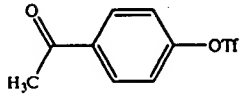
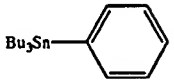
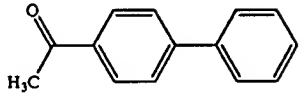
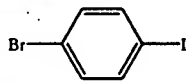
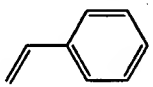
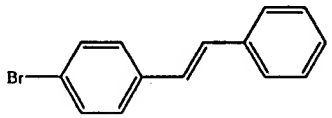
entry	aryl halide or aryl triflate	olefine or organometallic	time effect	product	isolated yield[a]	conventional heating time (min)	conventional heating yield (%)
6			2.50 min. 55 W $Pd(OAc)_2$, DPPP DMF, H_2O		77%		
7			7.00 min. 35 W $Pd(OAc)_2$, Ph_3P DMF		87%	540	93%[12]
8			5.00 min. 50 W $Pd(OAc)_2$, DPPF CH_3CN		54%	960	59%
9			5.00 min. 50 W $Pd(OAc)_2$, DPPF CH_3CN		64%	2880	72%
10			2.50 min. 55 W $Pd(PPh_3)_4$, EtOH, DME, H_2O		55%	360	94%[13]
11			2.50 min. 50 W $Pd_2(dba)_3$, Ph_3As, LiCl, NMP		68%	8400	82%[14]

TABLE 1-continued

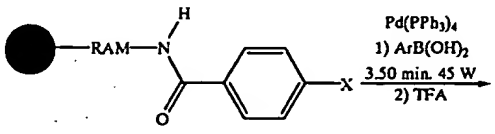
Palladium-Catalyzed Coupling Reactions in Solution under Microwave Irradiation

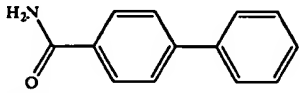
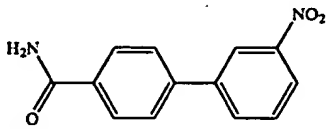
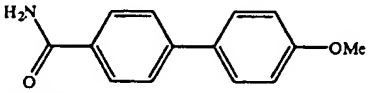
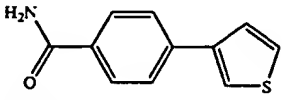
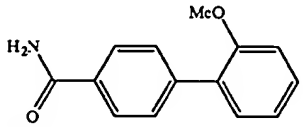
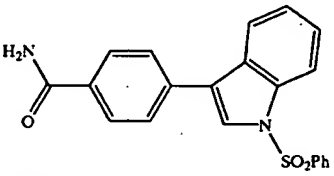
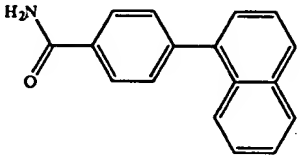
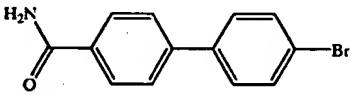
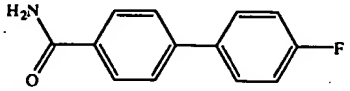
entry	aryl halide or aryl triflate	olefine or organometallic	time effect	product	isolated yield[a]	conventional heating: time (min)	conventional heating: yield (%)
12			4.50 min. 60 W → $Pd(OAc)_2$, DMF		63%	1020	64%[15]

[a]Purity > 95% by GC/MS

TABLE 2

Suzuki Coupling Reactions on Solid Phase under Microwave Irradiation[a]

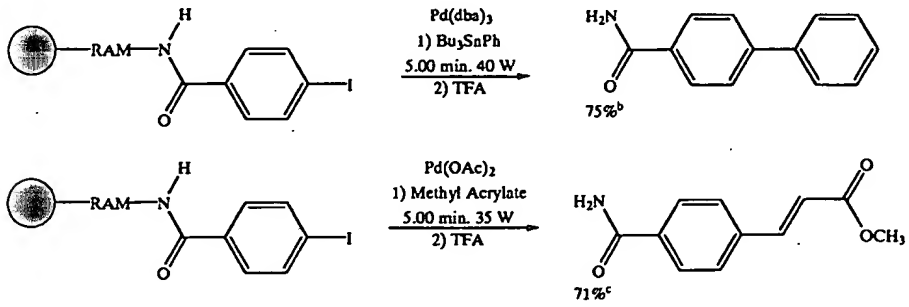


Entry		Entry	
1,2	 98%; X = I 98%; X = Br	10	 89%; X = I
	98%; X = I 98%; X = Br		89%; X = I
3,4	 96%; X = I 95%; X = Br	11,12	 86%; X = I 84%; X = Br
	96%; X = I 95%; X = Br		86%; X = I 84%; X = Br
5	 88%; X = I	13,14	 89%; X = I 88%; X = Br
	88%; X = I		89%; X = I 88%; X = Br
6,7	 99%; X = I 93%; X = Br	15[b]	 90%; X = I
	99%; X = I 93%; X = Br		90%; X = I
8,9	 99%; X = I 96%; X = Br		
	99%; X = I 96%; X = Br		

[a]Isolated yield (based upon the capacity of the Fmoc TentaGel S RAM-resin). Determined after correction (by 1H NMR) for the released PEG in the cleavage step. Purity > 95% by GC/MS.
[b]Irradiated for 6.00 min at 30 W. Purity > 80% by GC/MS.

TABLE 3

Stille and Heck Coupling Reactions on Solid Phase under Microwave Irradiation[a]



[a]Isolated yield (based upon the capacity of the Fmoc TentaGel S RAM-resin). Purity > 95% by GC/MS.
[b]The ratio of phenyl vs. butyl transfer in the isolated product was 9:1 according to ^{1}H NMR.
[c]76% conversion according to GC/MS.

COMPARISON OF PRODUCT PATTERN AFTER MICROWAVE AND STANDARD HEATING. IN SOLUTION:

The product pattern after microwave assisted palladium-catalyzed reactions and after standard heating was compared. For the reactions, entry 4 and entry 5 (see TABLE 1 above), were selected for more detailed studies. For the standard heating experiments, there was an optimal initial conversion rate of the arylhalides at 150–170° C. The reaction in entry 4, which represents the most simple system in TABLE 1, is performed with no added ligand and produces a stable product, and can be conducted at 150° C. in approximately the same reaction times as with microwave technology. A minimum amount of side products are formed with both heating techniques. The reaction in entry 5 represents a more complex system. Standard heating procedures (125–150° C.) produce here a very complex product mixture containing less than 20% of the desired product.

ON SOLID SUPPORT:

We have compared the product pattern after microwave assisted palladium-catalyzed reactions and after standard heating. Standard heating over 170° C. is not compatible with the solid support.

EXPERIMENTAL PART

(E)-Methyl4-methoxycinnamate. Table 1, Entry 1. In the reaction tube were mixed 4-iodoanisole (0.234 g, 1.0 mmol), methyl acrylate (0.108 g, 1.25 mmol), palladium acetate (0.00225 g, 0.01 mmol), tri-n-butylamine (0.185 g, 1.0 mmol) and 0.50 ml DMF.under nitrogen. The contents of the flask were irradiated for 3.50 min with an effect of 60 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml DCM. The combined extracts were washed with 25 ml water and concentrated to an oil. The crude product was purified by column chromatography on silica gel using i-hexane/diethyl ether (4/1) as the eluent. The yield was 70% (white solid).

(E)-Methyl-4-cyanocinnamate. Table 1, Entry 2. In the reaction tube were mixed 4-bromobenzonitrile (0.182 g, 1.0 mmol), methyl acrylate (0.108 g, 1.25 mmol), palladium acetate (0.00225 g, 0.01 mmol), tri-o-tolylphosphine (0.0122 g, 0.04 mmol), triethylamine (0.127 g, 1.25 mmol) and 0.50 ml DMF.under nitrogen. The contents of the flask were irradiated for 3.50 min with an effect of 60 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml DCM. The combined extracts were washed with 25 ml water and concentrated to an oil. The crude product was purified by column chromatography on silica gel using i-hexane/diethyl ether (1/1) as the eluent. The yield was 94% (pale yellow crystals).

(E)-Stilbene. Table 1, Entry 3. In the reaction tube were mixed iodobenzene (0.204 g, 1.0 mmol), styrene (0.130 g, 1.25 mmol), palladium acetate (0.00225 g, 0.01 mmol), tri-n-butylamine (0.185 g, 1.0 mmol) and 0.50 ml DMF.under nitrogen. The contents of the flask were irradiated for 2.50 min with an effect of 90 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml DCM. The combined extracts were washed with 25 ml water and concentrated to an oil. The crude product was purified by column chromatography on silica gel using i-hexane as the eluent. The yield was 87% (white crystals, terminal/internal arylation 19/1).

1-Naphthylacrylonitrile. Table 1, Entry 4. In the reaction tube were mixed 1-iodonaphthalene (0.254 g, 1.0 mmol), acrylonitrile (0.066 g, 1.25 mmol), palladium acetate (0.00225 g, 0.01 mmol), triethylamine (0.50 ml) and 0.50 ml DMF.under nitrogen. The contents of the flask were irradiated for 3.50 min with an effect of 80 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml DCM. The combined extracts were washed with 25 ml water and concentrated to an oil. The crude product was purified by column chromatography on silica gel using i-hexane/diethyl ether (2/1) as the eluent. The yield was 90% (white solid, trans/cis 4/1).

2-Phenyl-2,3-dihydrofuran. Table 1, Entry 5. In the reaction tube were mixed iodobenzene (0.102 g, 0.5 mmol), dihydrofuran (0.350 g, 5.0 mmol), palladium acetate (0.00281 g, 0.0125 mmol), potassium acetate (0.147 g,1.5 mmol), n-Bu_4NCl (0.139 g, 0.5 mmol), triphenylphosphine (0.00328 g, 0.0125 mmol) and 1.0 ml DMF.under nitrogen. The contents of the flask were irradiated for 6.00 min with an effect of 60 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml DCM. The combined extracts were washed with 25 ml water and concentrated to an oil. The crude product was purified by column chromatography on silica gel using pentaneldiethyl ether (19/1) as the eluent. The yield was 58% (yellow oil).

4-t-Butylacetophenone. Table 1, Entry 6. In the reaction tube were mixed 4-t-butylphenyl triflate (0.282 g, 1.0 mmol), butyl vinyl ether (0.25 g, 2.5 mmol), palladium acetate (0.00561 g, 0.025 mmol), DPPP (0.0206 g, 0.050 mmol) triethylamine (0.121 g, 1.2 mmol) 0.075 ml water and 0.75 ml DMF.under nitrogen. The contents of the flask were irradiated for 2.50 min with an effect of 55 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml diethyl ether. The combined extracts were washed two times with 25 ml saline and concentrated to an oil. The crude product was purified by column chromatography on silica gel using i-hexane/diethyl ether (7/1) as the eluent. The yield was 77% (clear oil).

N,N-Dimethyl-2-[2-(2-naphthyl)ethenyloxy]ethanamine. Table 1, Entry 7. In the reaction tube were mixed 2-naphthyl triflate (0.276 g, 1.0 mmol), [2-(dimethylamino)ethoxy] ethene (0.23 g, 2.0 mmol), palladium acetate (0.00673 g, 0.030 mmol), triphenylphosphine (0.0173 g, 0.066 mmol) triethylamine (0.202 g, 2.0 mmol) and 0.75 ml DMF.under nitrogen. The contents of the flask were irradiated for 7.00 min with an effect of 35 W. After cooling, the product mixture was diluted with 50 ml pentane, transferred to a separatory funnel and washed with 2×25 ml water. Additional extraction of the aqueous phases was performed with 25 ml pentane. The combined organic portions were then treated 5 times with 20 ml of 0.1 M HCl. The aqueous extracts were combined, poured into a flask containing excess NaOH (1.0 M) and was extracted with 2×50 ml pentane. The combined organic phases were dried (K_2CO_3) and concentrated by evaporation. The yield was 87% (yellow oil, trans/cis 1/1).

2-(4-Cyanophenyl)allyltrimethylsilane. Table 1, Entry 8. In the reaction tube were mixed 4-cyanophenyl triflate (0.251 g,1.0 mmol), allyltrimethylsilane (0.571 g, 5.0 mmol), palladium acetate (0.00673 g, 0.030 mmol), DPPF (0.0366 g, 0.066 mmol), potassium carbonate (0.207 g, 1.5 mmol) and 1.0 ml acetonitrile.under nitrogen. The contents of the flask were irradiated for 5.00 min with an effect of 50 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml diethyl ether. The combined extracts were washed two times with 25 ml saline and concentrated to an oil. The crude product was purified by repeated Kugelrohr distillation. The yield was 54% (clear oil).

2-(2-Naphthyl)N-allyidimethylamine. Table 1, Entry 9. In the reaction tube were mixed 2-naphthyl triflate (0.276 g, 1.0 mmol), N-allyidimethylamine (0.426 g, 5.0 mmol), palladium acetate (0.00673 g, 0.030 mmol), DPPF (0.0366 g, 0.066 mmol), potassium carbonate (0.207 g, 1.5 mmol) and 1.0 ml acetonitrile.under nitrogen. The contents of the flask were irradiated for 5.00 min with an effect of 50 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml diethyl ether. The combined extracts were washed two times with 25 ml saline and concentrated to an oil. The crude product was purified by repeated Kugelrohr distillation. The yield was 64% (clear oil).

4-Methylbiphenyl. Table 1, Entry.10. In the reaction tube were mixed 4-bromotoluene (0.171 g, 1.0 mmol), phenylboronic acid (0.134 g, 1.1 mmol), $Pd(PPh_3)_4$ (0.0347 g, 0.030 mmol), sodium cabonate (0.212 g, 2.0 mmol), 0.187 ml 95% ethanol, 0.375 ml water and 0.75 ml DME.under nitrogen. The contents of the flask were irradiated for 2.50 min with an effect of 55 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml DCM. The combined extracts were washed with 25 ml saline and concentrated to an oil. The crude product was purified by column chromatography on silica gel using i-hexane as the eluent. The yield was 55% (white solid).

4'-Phenylacetophenone. Table 1, Entry 11. In the reaction tube were mixed 4-acetylphenyl triflate (0.268 g, 1.0 mmol), phenyltributyltin (0.441 g, 1.2 mmol), Pd_2dba_3 (0.00916 g, 0.010 mmol), triphenylarsine (0.0245 g, 0.080 mmol), lithium chloride (0.127 g, 3.0 mmol) and 1.0 ml NMP.under nitrogen. The contents of the flask were irradiated for 2.50 min with an effect of 50 W. After cooling, addition of 1 M aqueous KF (4 ml) with stirring for 120 min, were followed by dilution (DCM) and filtration through a pad of celite. The filtrate was washed with water (25 ml). Additional extraction of the aqueous phase was performed with DCM (2×25 ml). The combined extracts were washed with 25 ml saline and concentrated to an oil. The crude product was purified by column chromatography on silica gel using i-hexane/ethyl acetate (10/1) as the eluent. The yield was 68% (white solid).

(E)4-Bromostilbene. Table 1, Entry 12. In the reaction tube were mixed 4-bromoiodobenzene (0.283 g, 1.0 mmol), styrene (0.115 g, 1.1 mmol), palladium acetate (0.00225 g, 0.01 mmol), triethylamine (0.111 g, 1.1 mmol) and 0.50 ml DMF.under nitrogen. The contents of the flask were irradiated for 4.50 min with an effect of 60 W. After cooling, the product mixture was poured into 25 ml water and was extracted three times with 25 ml DCM. The combined extracts were washed with 25 ml water and concentrated to an oil. The crude product was purified by column chromatography on silica gel using i-hexane as the eluent. The yield was 63% (white crystals).

General procedure for Suzuki coupling reactions on solid phase. Table 2. A sealed Pyrex tube was charged, under nitrogen, with 4-iodo or 4-bromo functionalised resin (100 mg, loading ~0.23 mmol/g), $Pd(PPh_3)_4$ (1.15 mg, 0.0010 mmol), aryl boronic acid (0.20 mmol), 2M Na_2CO_3 (0.10 ml, 0.20 mmol), H_2O (0.30 ml), ethanol (0.19 ml) and DME (0.75 ml). After irradiation, the mixture was cooled to room temperature. The resin was transferred thereafter to a 3-ml disposable syringe equipped with a porous polyethylene filter using H_2O/DME, washed with successive portions of H_2O, DME, DMF, sat. KCN/DMSO, MeOH, H_2O, MeOH and DCM (2×3 ml each) and dried. The resin was treated with 99% aq. TFA for 1 h, filtered and washed with 0.5 ml TFA and 2×0.5 ml DCM. The filtrates were combined and evaporated to dryness to yield the biaryl. All products gave satisfactory ^1H NMR spectra as well as appropriate ion identification by mass spectrometry (EI or PDMS) and had purity >95% by GC/MS.

Procedure for the Stille coupling reaction on solid phase. Table 3. 4-Iodo functionaiised resin (100 mg, loading ~0.23 mmol/g), Pd_2dba_3 (1.05 mg, 0.00115 mmol), $AsPh_3$ (1.41 mg, 0.0046 mmol), phenyltributyltin (73.4 mg, 0.20 mmol) and dry NMP (1.0 ml) were placed in a Pyrex tube under nitrogen. The tube was closed, placed in the microwave cavity and irradiated for 5.00 min. at 40 W. After cooling, the product was washed and cleaved from the resin as described for the Suzuki coupling reactions. The ratio of phenyl vs. butyl transfer in the isolated product was 9:1 according to ^1H-NMR (75% yield, white solid).

Procedure for the Heck coupling reaction on solid phase. Table 3. 4-Iodo functionalised resin (50 mg, loading ~0.23 mmol/g), methyl acrylate (0.046 g, 0.53 mmol), palladium acetate (0.00074 g, 0.0033 mmol), tri-o-tolylphosphine (0.00322 g, 0.0106 mmol), triethylamine (0.011 g, 0.11 mmol) and 1.0 ml DMF were placed in a Pyrex tube under

nitrogen. The tube was closed, placed in the microwave cavity and irradiated for 5.00 min. at 35 W. After cooling, the product was washed and cleaved from the resin as described for the Suzuki coupling reactions. The crude product was purified by column chromatography on silica gel using i-hexane/DCM (1/1) as the eluent. The yield was 71% (white solid).

In the Heck, Suzuki and Stille reactions performed with microwave energy according to the invention catalytic systems commonly used in these reactions are employed. Below a listing of suitable catalytic systems of soluble palladium complexes will follow. The listed catalytic systems are not the active catalysts in the reactions but rather precatalysts generating the active catalyst.

$Pd(O)(PPh_3)_4$

$Pd(II)Cl_2(PPh_3)_2$

$Pd(II)OAc_2$+ligand (eg. PPh_3, $AsPh_3$, tri(2-furyl)phosphine, tri (o-tolyl) phosphine, DPPF (1,1'-bis (diphenylphosphino)ferrocene or BINAP (2,2'-bis (diphenylphosphino)-1,1'-binaphtyl)

$Pd(O)_2(dba)_3$—$CHCl_3$ or $Pd(O)(dba)_2$+ligand as above

Often additions are made to the catalytic systems, such as LiCl, Bu_4NCl, Cu salts, Ag salts, or Tl salts.

Furthermore, it is to be understood that the microwave energy used in the method according to the present invention can be generated in different ways. The microwaves should be generated in a controlled and most of all reproducible way. For large volumes (>0.2 mL), this can be achieved by using magnetrons or clystrons. For small reaction volumes (approx. 100 μl) semiconductor generated microwaves are preferably used.

The standing wave, single mode, performance of the applied microwaves makes it possible to focus and optimise the coupling of the microwaves to the reaction samples in a highly reproducible way.

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18. The 4-halobenzoic acids (4 eq.) were coupled to deprotected resin using 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate (HBTU, 4 eq.) and DIEA (8 eq.) for 2 h. Remaining amino groups were then capped by acetylation

19. 90 mm Fmoc TentaGel S RAM-resin (S30 023, 0.23 mmol/g capacity) purchased from Rapp Polymere.

What is claimed is:

1. A method for generating palladium-catalyzed organic reaction products, comprising performing an organic reaction catalyzed with palladium except Pd/C, the organic reaction being

i) Heck reactions in which a first organic species, Org^1X, is reacted with a second organic species, H-Olefin, in order to provide a product, Org^1-Olefin,

ii) Stille reactions in which a first organic species, Org^1X, is reacted with a second organic species, $R^1{}_3Sn\text{-}Org^2$, in order to provide a product, $Org^1\text{-}Org^2$, or

iii) Suzuki reactions in which a first organic species, Org^1X, is reacted with a second organic species, $R^2{}_2B\text{-}Org^3$, in order to provide a product, $Org^1\text{-}Org^3$,

wherein:

Org^1 is aryl, heteroaryl, vinyl, acetylenyl, alkyl, allyl, benzyl, acyl, or benzoyl, or mono- or polysubstituted aryl, heteroaryl, vinyl, acetylenyl, alkyl, allyl, or benzoyl;

X is a halide, triflate, mesitylate, nonaflate, carbonylhalide, sulfonylhalide, perfluoroalkylsulfonate, arylphosphate, alkylphosphate, diarylarsine, diarylphosphine, diarylstibine, aryliodonium salt or diazonium salt;

H-Olefin is an olefin having a double bond and an olefinic hydrogen atom, the double bond in the H-Olefin being unsubstituted or mono-, di- or trisubstituted;

R^1 is alkyl, aryl or heteroaryl, or mono- or polysubstituted alkyl, aryl or heteroaryl;

Org^2 and Org^3 are both aryl, heteroaryl, vinyl, acetylenyl, alkyl, allyl or benzyl, or mono- or polysubstituted aryl, heteroaryl, vinyl, acetylenyl, alkyl, allyl or benzoyl; and

R^2 is hydroxy, alkoxy, aryloxy or heteroaryloxy;

wherein microwave energy is supplied to the organic reaction in order to heat said organic reaction.

2. A method according to claim 1, wherein the organic reaction is the Heck reaction.

3. A method according to claim 2, wherein the organic reaction is performed in solution.

4. A method according to claim 2, wherein the organic reaction is performed on a solid support.

5. A method according to claim 4, wherein the first organic species, Org^1, or the second organic species, H-Olefin, is attached to the solid support.

6. A method according to claim 5, wherein the first organic species, Org^1, or the second organic species, H-Olefin, is attached to the solid support via a linker.

7. A method according to claim 2, wherein the organic reaction is part of a combinatorial chemistry process.

8. A method according to claim 1, wherein the organic reaction is the Stille reaction.

9. A method according to claim 8, wherein the organic reaction is performed in solution.

10. A method according to claim 8, wherein the organic reaction is performed on a solid support.

11. A method according to claim 10, wherein the first organic species, Org^1, or the second organic species, Org^2, is attached to the solid support.

12. A method according to claim 11, wherein the first organic species, Org^1, or the second organic species, Org^2, is attached to the solid support via a linker.

13. A method according to claim 8, wherein the organic reaction is part of a combinatorial chemistry process.

14. A method according to claim 1, wherein the organic reaction is the Suzuki reaction.

15. A method according to claim 14, wherein the organic reaction is performed in solution.

16. A method according to claim 14, wherein the organic reaction is performed on a solid support.

17. A method according to claim 15, wherein the first organic species, Org^1, is attached to the solid support.

18. A method according to claim 17, wherein the first organic species, Org^1, is attached to the solid support via a linker.

19. A method according to claim 14, wherein the organic reaction is part of a combinatorial chemistry process.

20. A method according to claim 1, wherein the microwave energy is solely or predominantly provided in the form of a standing wave.

21. A method according to claim 1, wherein the organic reaction is used in the creation of a chemical library.

22. A method according to claim 1, wherein the microwave energy is provided for a period of 2–7 minutes.

* * * * *

Nucleic Acid Related Compounds. 39. Efficient Conversion of 5-Iodo to 5-Alkynyl and Derived 5-Substituted Uracil Bases and Nucleosides[1]

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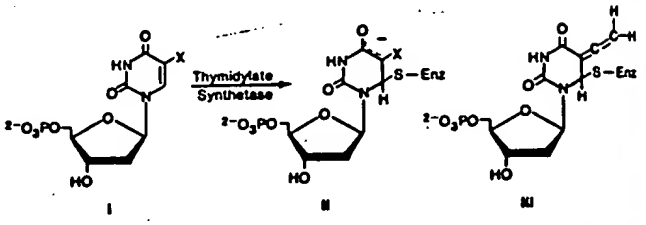
Coupling of terminal alkynes with 5-iodo-1-methyluracil and 5-iodouracil nucleosides (protected as their *p*-toluyl esters) proceeded in high yields in the presence of bis(triphenylphosphine)palladium(II) chloride and copper(I) iodide in warm triethylamine. Several of the subsequently deprotected 5-alkynyl-2′-deoxyuridines, including the parent 5-ethynyl-2′-deoxyuridine, had antiviral activity, and their 5′-monophosphates inhibited thymidylate synthetase. Hydrogenation of the 5-alkynyl side chain can be controlled to give (*Z*)-5-alkenyl- or the saturated 5-alkyl-2′-deoxyuridines. This provides a stereocontrolled route to the known 5-ethyl- and 5-*n*-hexyl-2′-deoxyuridines as well as (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU). Hydration of the triple bond gave the corresponding uracil-5-alkanone products in favorable cases.

A broad spectrum of biological activity has been described for 5-substituted uracil bases and nucleosides. The well-known cancer chemotherapeutic drug 5-fluorouracil and antiviral agents 5-iodo-2′-deoxyuridine and 5-(trifluoromethyl)-2′-deoxyuridine have been in clinical use for a number of years. More recently a variety of 5-substituted 2′-deoxyuridine derivatives have been synthesized and evaluated as antiherpes agents.[3] One highly potent and selective antiviral drug of this class, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU),[4] is presently undergoing clinical evaluation. The herpes simplex genome codes for a thymidine kinase with a substrate tolerance that readily accepts 5-substituents as large as the 2-bromoethenyl side chain.[5]

Pronounced cytotoxicity and significant antiviral activity have been reported for 5-ethynyl-2′-deoxyuridine.[4,6,7] Inhibition of thymidylate synthetase by 5-ethynyl-2′-deoxyuridylate also has been noted.[6,8,9] Enzymes including 2′-deoxyuridylate hydroxymethylase[10] and aminoacyl transfer ribonucleic acid synthetases[11] as well as the extensively studied thymidylate synthetase[12] are thought to function by initial Michael attack of an enzyme-bound nucleophile (probably the sulfhydryl group of a cysteine) at C6 of the enone system of uracil. Attack of the enolate anion of the initial adduct (ii, X = F) of 5-fluoro-2′-deoxyuridylate (i, X = F) with thymidylate synthetase occurs on $N^{5,10}$-methylenetetrahydrofolate to give a covalent ternary complex (enzyme–drug–cofactor).[12] Michael



attack of the enzyme at C6 of 5-ethynyl-2′-deoxyuridylate (i, X = C≡CH) has been postulated to result in transient formation of a highly reactive α-keto allene system (iii).[8,9,13]

Syntheses of 5-ethynyl-2′-deoxyuridine had previously involved construction of the heterocycle, coupling with a functionalized 2-deoxy sugar, and separation of the resulting anomeric mixture.[6,14] We now report details of a convenient and high-yield coupling procedure[1b] that provides direct access to 5-alkynyluracil bases and nucleosides from terminal alkynes and readily available[15] 5-iodouracil derivatives. Several of these 5-alkynyluracil nucleosides have antiviral activity,[16] and the derived 5-alkynyl-2′-deoxyuridylates inhibit thymidylate synthetase.[9] Transformations of the alkynyl side chain have been explored briefly to give BVDU and other alkenyl, alkyl, and alkanone substitutents.

Bergstrom[17] first applied the palladium-catalyzed coupling of alkenes with 5-mercuri or 5-iodo derivatives of uracil nucleosides, on the basis of the pioneering studies of Heck.[18] Daves,[19] Jones and Walker,[20] Mertes,[21] and their co-workers have since reported syntheses of C5-linked uracil nucleosides with carbon side chains based on these precedents. Bergstrom had reported[17b] that attempts to couple 5-chloromercuri- or 5-iodouridine with phenylacetylene by using Heck's procedures gave starting uridine or a complex mixture. Sonogashira et al.[22] had described a modified approach by including a copper(I) catalyst for coupling terminal alkynes with aryl and vinyl halides. Edo

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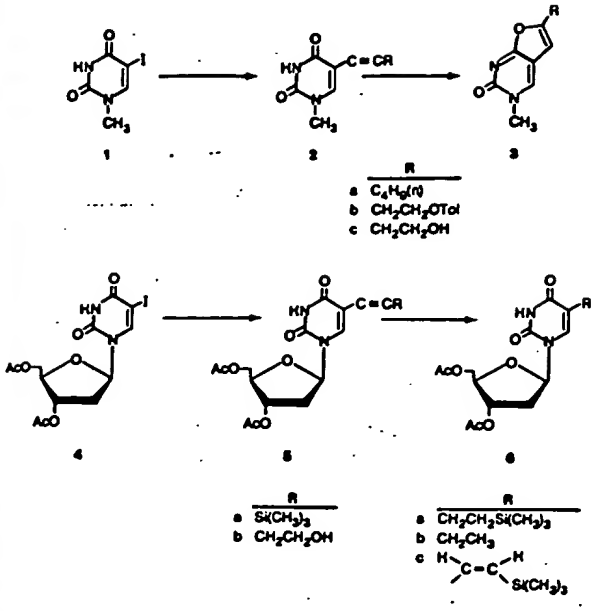
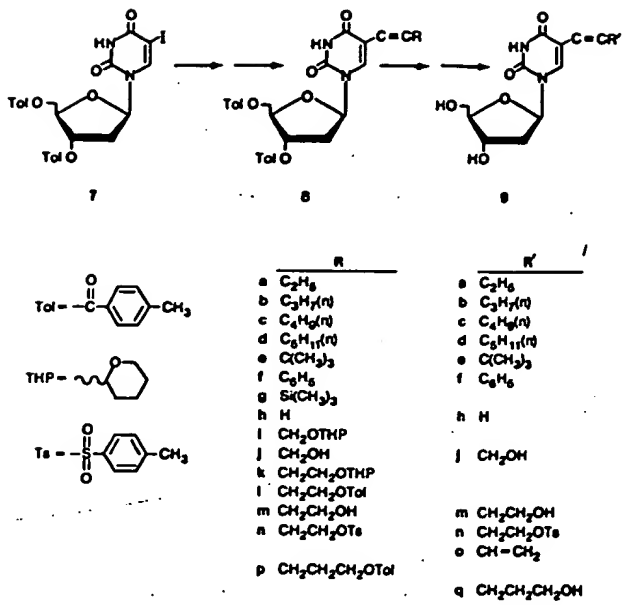
APPLICANT'S EXHIBIT B

Scheme I



Scheme II



and co-workers[23] had applied this method to substituted nitrogen heterocycles. Our adaptation of this general procedure allowed smooth and efficient coupling of terminal alkynes with 5-iodo-1-methyluracil and protected 5-iodouracil nucleosides under mild conditions.[1b] A more complex coupling sequence that results in variable product yields has since been noted by Pichat.[24]

Treatment of 5-iodo-1-methyluracil (1) with hexyne[1c] in triethylamine at 50 °C for 2 h in the presence of bis(triphenylphosphine)palladium(II) chloride and copper(I) iodide under a nitrogen atmosphere gave an 84% yield of 5-hexynyl-1-methyluracil[1c] (2a) (see Scheme I) after processing and column chromatography over silica. A 9% yield of the fluorescent 6-*n*-butyl-3-methylfurano[2,3-*d*]-pyrimidin-2-one (3a) was recovered from later column fractions. Treatment of 2a with CuI in triethylamine/methanol at reflux gave 3a in 92% yield. Similar cyclizations have been noted in Stephens–Castro couplings of *o*-iodoanilines with copper(I) acetylides[25] and by treatment of (*E*)-5-(2-bromovinyl)uracil with strong base.[26]

The extent of cyclization was markedly dependent on the reactants. A 77% yield of 1-methyl-5-[4-(*p*-toluyloxy)butynyl]uracil (2b) was obtained by direct crystallization of the product from coupling 1 with 4-(*p*-toluyloxy)butyne. Only a minor amount of the fluorescent byproduct (presumably 3b) was observed. Coupling of 5-iodo-3′,5′-di-*O*-acetyl-2′-deoxyuridine (4) with (trimethylsilyl)acetylene gave 5-[(trimethylsilyl)ethynyl]-3′,5′-di-*O*-acetyl-2′-deoxyuridine (5a) in 80% yield. However, coupling of 4 with hexyne, 4-(*p*-toluyloxy)butyne, and 4-(tetrahydropyranyloxy)- or 4-(trityloxy)butyne under the same conditions gave the cyclized furano[2,3-*d*]pyrimidin-2-one compounds as major products. Thin-layer chromatography indicated that cyclization of the 5-alkynyluracil products also occurred during workup. This was moderated by addition of solid disodium EDTA to the warm coupling mixture before processing. This modification was employed in a coupling of 4 with 4-(trityloxy)butyne. Detritylation and chromatographic analysis gave starting 4 (7%), deiodinated 4 (3′,5′-di-*O*-acetyl-2′-deoxyuridine, 11%), product 5-(4-hydroxybutynyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (5b, 61%), and cyclized furano[2,3-*d*]pyrimidin-2-one (9%).

Protection of nucleoside hydroxyl groups as *p*-toluyl esters provided organic-soluble and easily crystallized products in ~95% yields. We have recently described procedures for high-yield iodination (and chlorination) of uracil compounds that gave 5-iodo-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (7; see Scheme II) in 98% crystallized yield.[15] Coupling reactions using 7 proceeded smoothly with minimal formation of deiodinated and furano[2,3-*d*]pyrimidin-2-one byproducts. Treatment of 7 with hexyne under the usual conditions gave 5-hexynyl-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (8c) in 89% purified yield.[27] Scheme II gives the structures and Table I lists characterization data for the 5-alkynyl-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine products.

Treatment of the 5-(trimethylsilyl)ethynyl compound (8g) with potassium fluoride/tetraethylammonium bromide effected removal of the trimethylsilyl group to give the known[14] 5-ethynyl-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (8h) in 84% yield. Complete deprotection of 8g occurred in 0.2 N sodium methoxide in dry methanol to give crude 5-ethynyl-2′-deoxyuridine[6,14] (9h) quantitatively. This provides a stereocontrolled sequence from 2′-deoxyuridine to 9h (~75% overall yield) that makes this compound accessible for further biological evaluation. Analogous deprotection of the other *p*-toluyl ester products (8) gave the 2′-deoxyuridine derivatives (9) noted in Scheme II and characterized by the data listed in Table II.

Protection of ω-hydroxyalkynes as tetrahydropyranyl (THP) acetals or *p*-toluyl esters gave derivatives that underwent smooth coupling with the 5-iodouracil compounds. Acid-catalyzed removal of the THP group from 8i and 8k gave the 5-(ω-hydroxyalkynyl)-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridines (8j and 8m, respectively). Treatment of 8m with *p*-toluenesulfonyl chloride/pyridine gave the ω-to-

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(27) Coupling of unprotected 5-iodouracil nucleosides with hexyne under the typical conditions did not proceed, and more strenuous treatment gave mixtures of products. Usual treatment of 5-bromo-3′,5′-di-*O*-acetyl-2′-deoxyuridine with hexyne resulted in a sluggish reaction. The 5-chlorouracil compounds tried did not undergo coupling with hexyne under the usual conditions.

Table I. Characterization Data for 5-Alkynyl-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine Intermediates

compd	mp, °C	yield, %	calcd C	calcd H	calcd N	found C	found H	found N	characteristic ^{1}H NMR spectral peaks,[a,b] δ
8a	224–225	91	c						1.04 (t, 3, CH$_2$CH$_3$), 2.30 (q, 2, CH$_2$CH$_3$), 2.40 (br s, 6, ArCH$_3$'s), 6.25 (t, 1, H1′)
b	c	85	c						0.94 (t, 3, CH$_2$CH$_3$), 1.44 (m, 2, CH$_2$CH$_3$), 2.28 (t, 2, C≡CCH$_2$), 2.40 (br s, 6, ArCH$_3$'s), 6.28 (dd, 1, H1′)
c	213–214	90	68.37	5.92	5.14	68.33	5.72	4.83	[d] 0.90 (m, 3, CH$_2$CH$_3$), 2.38 (s, 6, ArCH$_3$'s), 6.34 (dd, 1, H1′), 7.25 (d, 4, aromatic), 7.72 (s, 1, H6), 7.91 (d, 4, aromatic)
d	197–201	77	c						[d] 0.91 (m, 3, CH$_2$CH$_3$), 2.21 (t, 2, C≡CCH$_2$), 2.44 (s, 6, ArCH$_3$'s), 6.40 (dd, 1, H1′), 7.25 (d, 4, aromatic), 7.78 (s, 1, H6), 7.95 (d, 4, aromatic)
e	228–231	91	c						1.12 (s, 9, C(CH$_3$)$_3$), 2.40 (br s, 6, ArCH$_3$'s), 6.23 (t, 1, H1′)
f	c	91	c						2.26 and 2.40 (s and s, 3 and 3, ArCH$_3$'s), 6.30 (t, 1, H1′), 7.2–7.9 (m, 13, aromatic), 8.09 (s, 1, H6)
g	255–256	85	64.27	5.75	5.00	64.22	5.62	4.94	[e] 0.12 (s, 9, Si(CH$_3$)$_3$), 2.40 (br s, 6, ArCH$_3$'s), 6.30 (dd, 1, H1′)
i	f	72	65.77	5.69	4.65	65.75	5.56	4.62	2.40 (s, 6, ArCH$_3$'s), 4.31 (dd, 2, C≡CCH$_2$), 6.25 (t, 1, H1′) 8.02 (s, 1, H6)
j	194–197	90	64.86	5.05	5.40	64.54	4.99	5.24	2.40 (s, 6, ArCH$_3$'s), 4.19 (s, 2, C≡CCH$_2$), 6.26 (t, 1, H1′), 8.00 (s, 1, H6)
k	164–165	85	66.22	5.88	4.54	65.75	6.00	4.53	[f] 2.39 (br s, 6, ArCH$_3$'s), 6.23 (t, 1, H1′)
l	f	85	68.30	5.27	4.31	67.99	5.22	4.56	[d] 2.41 (m, 9, ArCH$_3$'s), 2.66 (t, 2, C≡CCH$_2$), 4.28 (t, 2, CH$_2$O), 6.40 (dd, 1, H1′)
m	236–238	90	65.41	5.30	5.26	65.33	5.36	5.14	2.40 (m, 8, ArCH$_3$'s and C≡CCH$_2$), 3.49 (m, 2, CH$_2$OH), 4.80 (t, 1, CH$_2$OH), 6.28 (dd, 1, H1′)
n	f	65	62.96	4.99	4.08	62.54	5.32	4.01	2.40 (m, 9, ArCH$_3$'s), 2.66 (t, 2, C≡CCH$_2$), 4.02 (t, 2, CH$_2$OTs), 6.28 (t, 1, H1′)
p	f	87	68.66	5.46	4.21	68.35	5.53	4.09	1.86 (m, 2, CH$_2$CH$_2$CH$_2$), 2.37, 2.39, 2.41 (3 s, 3, 3, and 3, ArCH$_3$'s), 2.48 (t, 2, C≡CCH$_2$), 4.32 (t, 2, CH$_2$OTol), 6.30 (t, 1, H1′)

[a] Spectra were determined at 100 MHz in Me$_2$SO-d_6 with Me$_4$Si as internal standard unless otherwise noted. [b] Unresolved multiplets with extensive overlap for most sugar protons (and some side-chain protons), the aromatic protons (sometimes overlapping H6), and the broad singlet for NH at δ 9–12 are not listed. [c] Deprotected without further characterization. [d] CDCl$_3$. [e] CDCl$_3$/Me$_2$SO-d_6. [f] Softening with no distinct melting range. [g] 200 MHz.

syloxy product (8n). Deprotection of 8n with 0.1 N NaOMe/MeOH gave 9n. Treatment of 8n with potassium *tert*-butoxide in acetonitrile followed by methanol gave 5-(but-3-en-1-ynyl)-2′-deoxyuridine (9o).

An example of a conjugated ynene system attached in the reverse orientation at C5 was obtained by coupling hexyne with the vinologous bromo acyclonucleoside analogue 1-[(2-acetoxyethoxy)methyl]-(*E*)-5-(2-bromovinyl)-uracil[28] (10). The resulting product was deacetylated to

10 11

give 1-[(2-hydroxyethoxy)methyl]-(*E*)-5-(oct-1-en-3-ynyl)uracil (11). Retention of the *E* stereochemistry was indicated by the large vinylic coupling constant (J = 16 Hz) measured at 400-MHz resolution.

The presently described synthesis of 5-alkynyluracil products presented a potentially facile route to known biologically active 5-alkyl- and -alkenyl-2′-deoxyuridines. Hydrogenation of 5-ethynyl-2′-deoxyuridine (9h) over palladium on carbon gave the known antiviral agent 5-ethyl-2′-deoxyuridine[29] (12) in 93% yield. Similarly efficient reduction of the 5-hexynyl compound (9c) gave 5-*n*-hexyl-2′-deoxyuridine[30a] (13). Cytostatic activity in mammalian cells had been reported for 13.[30b] However, a minimal activity response was seen with our pure 13 in the standard murine leukemia L1210 cell system.[16] Hydrogenation of 5-[(trimethylsilyl)ethynyl]-3′,5′-di-*O*-acetyl-2′-deoxyuridine (5a) gave 5-[(trimethylsilyl)ethyl]-(6a) plus 5-ethyl-3′,5′-di-*O*-acetyl-2′-deoxyuridine (6b) in 67% and 23% yields, respectively.

Careful partial hydrogenation of the 5-hexynyl compound 9c over Lindlar catalyst with quinoline in acetone gave 83% of (*Z*)-5-hexenyl-2′-deoxyuridine (14) plus 10%

12 C_2H_5
13 $C_6H_{13}(n)$
14
15
16
17 CH_3
18 R′=X=H
19 R′=H, X=OH
20 R′=Ac, X=H

of the saturated 5-*n*-hexyl compound 13. Separation of these products from a trace of starting 9c was effected by preparative reverse-phase HPLC. The smaller vinylic coupling constant (J = 11.5 Hz) at 400-MHz resolution was consistent with the cis geometry[31] for 14. A 12% NOE

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Table II. Characterization Data for 5-Alkynyl-2'-deoxyuridines

compd	mp, °C	UV pH 6 max, nm (ε)	UV pH 6 min, nm (ε)	UV pH 13 max, nm (ε)	UV pH 13 min, nm (ε)	calcd C	calcd H	calcd N	found C	found H	found N
9a[e]	178–179	293 (10 600), 232 (10 800)	258 (3000)	287 (8500), 230 (13 000)	262 (4500)	53.97	5.92	9.68	54.00	5.80	9.85
b[e]	139–144	292 (10 800), 230 (12 300)	257 (3200)	287 (8700), 230 (14 700)	260 (4600)	55.44	6.31	9.24	55.73	6.20	9.35
c	136–138	293 (11 700), 233 (11 600)	256 (3300)	287 (9300), 232 (12 800)	260 (4500)	58.43	6.54	9.09	58.21	6.52	8.88
d	130–132	293 (11 000), 234 (11 000)	259 (3000)	290 (9100), 233 (13 300)	264 (4600)	59.61	6.88	8.69	59.88	6.91	8.57
e[g]	104–109	292 (11 300), 232 (12 200)	258 (3600)	287 (9200), 229 (14 500)	260 (4700)	55.21	6.80	8.58	55.26	6.22	8.57
f	174–176	307 (16 300), 279 (12 200), 264 (13 300)	286 (12 200), 271 (12 100), 240 (9400)	305 (16 500), 282 (13 800), 266 (12 500)	287 (13 600), 271 (12 300), 246 (9400)	62.19	4.91	8.53	62.29	4.87	8.70
j	182–184	288 (11 000), 234 (12 700)	259 (4600)	287 (9600), 234 (12 700)	262 (4700)	51.06	5.00	9.92	50.89	4.97	9.87
m	163–165	293 (12 000), 233 (12 000)	257 (3500)	288 (9600), 228 (14 200)[h]	261 (4700)	52.70	5.44	9.46	52.39	5.68	9.29
n[g]	102–104	292 (13 500), 228 (24 800)	257 (4400)	287 (10 100), 228 (26 100)	262 (5700)	51.28	5.16	5.98	51.22	4.92	6.12
o[e]	>200 dec	301 (14 400) (8 other max)		298 (14 100) (3 other max)		54.35	5.26	9.75	54.47	4.98	9.54
q	163–164	292 (11 300), 232 (11 300)	257 (3400)	287 (9000) 232 (13 300)[h]	261 (4700)	54.19	5.85	9.03	54.05	6.01	9.07

compd	^{1}H NMR spectral peaks, δ, for nonexchangeable protons[a] H2',2''[b]	H5',5''[b]	H4'[b]	H3'[b]	H1'[c]	H6[d]	other peaks
9a[e]	2.12	3.60	3.80	4.24	6.12	8.12	1.12 (t, 3, CH_2CH_3), 2.38 (q, 2, CH_2CH_3)
b[e]	2.12	3.60	3.80	4.24	6.13	8.13	0.98 (t, 3, CH_2CH_3), 1.52 (m, 2, CH_2CH_3), 2.34 (t, 2, $C{\equiv}CCH_2$)
c	2.10	3.57	3.78	4.22	6.10	8.08	[f]0.88 (m, 3, CH_2CH_3), 1.44 (m, 4, $CH_2CH_2CH_3$), 2.32 (m, 2, $C{\equiv}CCH_2$)
d	2.11	3.58	3.79	4.22	6.10	8.08	[f]0.89 (m, 3, CH_2CH_3), 1.38 (m, 6, $CH_2CH_2CH_2CH_3$), 2.36 (m, 2, $C{\equiv}CCH_2$)
e[g]	2.10	3.59	3.78	4.24	6.09	8.06	[f]1.22 (s, 9, $C(CH_3)_3$)
f	2.17	3.62	3.82	4.26	6.14	8.40	7.4 (m, 5, phenyl)
j	2.12	3.59	3.80	4.24	6.13	8.20	4.24 (d, 2, $C{\equiv}CCH_2OH$)
m	2.10	3.55	3.80	4.24	6.14	8.14	2.50 (m, 2, $C{\equiv}CCH_2$), 3.55 (m, 2, CH_2OH)
n[g]	2.10	3.60	3.80	4.20	6.10	8.09	[f]2.40 (s, 3, $ArCH_3$), 2.74 (t, 2, $C{\equiv}CCH_2$), 4.20 (m, 2, CH_2OTs), 7.44 and 7.80 (A_2X_2, 4, aromatic)
o[e]	2.12	3.59	3.80	4.24	6.10[b]	8.27	5.67 (m, 2, $CH{=}CH_2$), 6.10 (m, $CH{=}CH_2$)
q	2.10	3.50	3.80	4.20	6.11	8.10	[f]1.60 (m, 2, $CH_2CH_2CH_3$), 2.50 (m, 2, $C{\equiv}CCH_2$), 3.50 (m, 2, CH_2OH)

[a] Spectra were determined in Me_2SO-d_6 with Me_4Si as an internal standard at 200 MHz unless otherwise noted. [b] Multiplet. [c] Apparent triplet. [d] Singlet. [e] Isolated as the hemihydrate. [f] 100 MHz. [g] Isolated as the monohydrate. [h] Shoulder.

enhancement of the vinyl 2-proton signal upon irradiation of the 1-proton corroborated this assignment. No *E* isomer was observed by analytical HPLC or in the 400-MHz ^{1}H NMR spectrum. Similar partial reduction of the 5-[(trimethylsilyl)ethynyl] compound 5a in ethyl accetate gave (*Z*)-5-[2-(trimethylsilyl)ethenyl]-3′,5′-di-*O*-acetyl-2′-deoxyuridine (6c) as the highly predominant product. The large vinylic coupling constant (J = 15 Hz) for 6c parallels data reported for the *E* and *Z* β-(trimethylsilyl)styrenes.[32] A 22% NOE enhancement of the vinyl 2-proton signal upon irradiation of the 1-proton at 400 MHz again corroborated the *Z* assignment for 6c.

Treatment of (*Z*)-β-(trimethylsilyl)styrene with bromine/carbon disulfide at −100 °C and processing with acetonitrile was reported to give mainly (*Z*)-β-bromostyrene.[33] Crude 6c was subjected to these conditions followed by deprotection with methanolic ammonia. However, the known antiviral agent (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (15, BVDU)[4,20] was obtained in 40% yield after purification by HPLC. This reaction was not examined further since the *Z* isomer of 15 had been obtained by this stage of our investigation.[34]

We briefly examined the conversion of certain 5-alkynyl to 5-alkanone compounds. Antiviral and thymidylate synthetase inhibitory activities have been reported for 5-formyl-2′-deoxyuridine and its 5′-phosphate, respectively.[35] Facile hydration of 5-ethynyl- (9h) to 5-acetyl-2′-deoxyuridine occurred under mild-acid conditions.[36] However, hydrophobic 5-alkynyl-2′-deoxyuridine compounds from the present study were resistant to hydration in acidic dioxane/water solutions. Prolonged treatment of 5-hexynyl-2′-deoxyuridine (9c) with mercury(II) sulfate in aqueous dioxane gave the cyclized 6-*n*-butyl-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)furano[2,3-*d*]pyrimidin-2-one (16) in 36% yield. Starting 9c and minor products were also present, but no significant quantity of the expected ketone was formed. Compound 16 was prepared in 82% yield by treatment of 9c with copper(I) iodide in hot triethylamine/methanol.

Treatment of 5-(4-hydroxybutynyl)-1-methyluracil (2c) with mercury(II) sulfate in aqueous solution at 50 °C for 2 h resulted in isolation of 5-(4-hydroxybutanoyl)-1-methyluracil (17) in 78% yield. A minor amount (~5%) of the cyclized furano[2,3-*d*]pyrimidin-2-one (3c) was also formed. Similar conversions of the 5-(4-hydroxybutynyl)-2′-deoxyuridine (9m) and uridine compounds to the corresponding ketones, 5-(4-hydroxybutanoyl)-2′-deoxyuridine (18) and uridine (19), were effected in 64% and 67% yields. However, treatment of 5-(4-hydroxybutynyl)-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (8m) with mercury(II) sulfate in aqueous dioxane resulted in reisolation of starting 8m. Treatment of 8m with mercury(II) acetate in hot trifluoroacetic acid resulted in loss of ultraviolet light absorption. The more water-soluble 5-(4-hydroxybutynyl)-3′,5′-di-*O*-acetyl-2′-deoxyuridine (5b) was hydrated to the 5-(4-hydroxybutanoyl) product 20 in 59% yield.

Conclusions

This study has provided a convenient and high-yield procedure for coupling terminal alkynes with 5-iodo-1-methyluracil and readily available[15] *p*-toluyl-protected 5-iodouracil nucleosides. A four-stage sequence from 2′-deoxyuridine to 5-ethynyl-2′-deoxyuridine was effected in ~75% overall yield. Hydrogenation of the alkynyl products can be controlled to give (*Z*)-5-alkenyl- or saturated 5-alkyluracil compounds. Previously reported 5-ethyl-, 5-*n*-hexyl-, and (*E*)-5-(2-bromovinyl)-2′-deoxyuridines (that have been prepared by base/sugar coupling as anomeric mixtures) were obtained with absolute anomeric purity on starting from naturally occurring 2′-deoxyuridine. Certain 5-(ω-hydroxyalkanoyl)uracil products were obtained by hydration of their ω-hydroxyalkynyl precursors. Antiviral[16] and thymidylate synthetase inhibitory activity[9] were found with certain of the 5-alkynyl-2′-deoxyuridine compounds.

Experimental Section

Melting points were determined on a Reichert microstage block and are uncorrected. Ultraviolet (UV) spectra were recorded on a Cary 15 spectrophotometer. The high-field NMR laboratory of this department obtained ^{1}H spectra on Varian 100 and Bruker WH-200 and WH-400 spectrometers with Me_4Si as an internal standard. Me_2SO-d_6 was used as the solvent, and spectra were determined at 100 MHz unless otherwise noted. Elemental analyses were determined by the microanalytical laboratory of this department or Schwarzkopf Microanalytical laboratory.

Thin-layer chromatography (TLC) was performed on Merck No. 5735 silica gel sheets by using the upper phase of *n*-PrOH/H_2O/EtOAc (1:2:4) as the developing solvent, unless otherwise noted, with sample detection under 2537-Å light. Preparative TLC was performed on Whatman PLK5F silica gel plates with a sample preloading zone. Column chromatography was effected by using J. T. Baker 5-3405 or Mallinckrodt CC-7 silica gel. Analytical HPLC was performed on a Whatman Partisil ODS-2 C-18 and preparative HPLC on a Waters Bondapak C-18 reverse-phase column by using acetonitrile/water at the indicated composition. Evaporations were effected at room temperature by using a Buchler rotary evaporator equipped with a Dewar dry-ice condenser under water-tap aspirator or mechanical oil pump vacuum.

Reagent grade pyridine and triethylamine were refluxed over and then distilled from calcium hydride. Reagent grade acetonitrile was refluxed over and distilled from phosphorus pentoxide. Anhydrous methanol was prepared by using magnesium turnings. Other reagent grade solvents were redistilled before use. "Hexane" refers to the fraction of petroleum ether (Skellysolve B) boiling from 63 to 65 °C upon redistillation. Copper(I) iodide was purchased from Fisher Scientific Co. Bis(triphenylphosphine)-palladium(II) chloride was prepared in 94% yield according to the procedure of Burmeister and Basolo.[37]

Alkynes were purchased from Farchan Division, Chemsampco Inc., and Petrarch Systems Inc. and were redistilled before use if necessary. Preparation of 3-(2-tetrahydropyranyloxy)propyne[38] and 4-(2-tetrahydropyranyloxy)butyne[39] followed the literature methods. One preparation of 4-(trityloxy)butyne was made by using trityl chloride and 3-butynol in pyridine followed by the usual processing and crsyallization of the product from ethanol. This material was used without further purification in one condensation reaction with 3′,5′-di-*O*-acetyl-5-iodo-2′-deoxyuridine (vide infra).

All coupling reactions were performed under an atmosphere of oxygen-free nitrogen, and the solvent (or solution) was vigorously deoxygenated with N_2 prior to addition of catalysts.

4-(*p*-Toluyloxy)butyne. A stirred solution of 14 g (0.2 mol) of 3-butynol in 100 mL of pyridine was cooled to 0 °C and treated with 34 g (0.22 mol) of *p*-toluyl chloride. The solution was warmed to 50 °C, stirred for 1 h, and evaporated. The residue was dissolved in 100 mL of $CHCl_3$, washed thoroughly with 1 M H_2SO_4/H_2O and then H_2O (2 × 50 mL), dried (Na_2SO_4), and

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evaporated to give a faintly yellow oil. This was distilled at 95–100 °C (0.5 mmHg) and then cooled to give 32.5 g (86%) of the title compound as colorless crystals. Anal. Calcd for $C_{12}H_{12}O_2$: C, 76.57; H, 6.43. Found: C, 76.63; H, 6.37.

5-(*p*-Toluyloxy)pentyne was prepared from 4-pentynol in a manner identical with that described above for its lower homologue. The title compound was obtained as a colorless oil, bp 110 °C (0.5 mmHg). Anal. Calcd for $C_{13}H_{14}O_2$: C, 77.20; H, 6.98. Found: C, 77.23; H, 7.00.

5-Hexynyl-1-methyluracil (2a). A suspension of 1.26 g (5 mmol) of 5-iodo-1-methyluracil[15] (1) in 60 mL of Et_3N was vigorously deoxygenated with oxygen-free nitrogen. Hexyne (820 mg, 10 mmol), $(Ph_3P)_2PdCl_2$ (25 mg), and CuI (25 mg) were added, and the suspension was stirred at 50 °C for 2 h under N_2. The mixture was evaporated thoroughly to dryness, and the residue was dissolved in 100 mL of $CHCl_3$. This solution was washed with 5% disodium EDTA/H_2O (2 × 50 mL), and 50 mL of H_2O, dried (Na_2SO_4), and evaporated to a small volume. This solution was applied to a column (100 g) of Mallinckrodt silica, and the column was developed with $CHCl_3$/MeOH (9:1). Evaporation of appropriate earlier fractions gave 864 mg (84%) of crystalline 2a. This was recrystallized from hexane/acetone to give 2a: mp 134–135 °C; NMR δ 0.90 (m, 3, CH_2CH_3) 1.50 (m, 4, $CH_2CH_2CH_3$), 2.40 (m, 2, $C{\equiv}CCH_2$), 3.22 (s, 3, NCH_3), 7.93 (s, 1, H6), 11.44 (br s, 1, NH). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 63.79; H, 6.85; N, 13.51.

Later fractions were pooled and evaporated to give 88 mg (9%) of 6-*n*-butyl-3-methylfurano[2,3*d*]pyrimidin-2-one (3a). This was recrystallized from 95% EtOH (with diffusion of Et_2O)[40] to give 3a: mp 190–192 °C (with sublimation at 179 °C); NMR δ 0.91 (m, 3, CH_2CH_3), 1.50 (m, 4, $CH_2CH_2CH_3$), 2.64 (t, 2, $ArCH_2$), 3.48 (s, 3, NCH_3), 6.40 (t, $^4J \approx 1$ Hz, 1, H5), 8.43 (s, 1, H4). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58. Found: C, 64.10; H, 6.76; N, 13.76.

6-*n*-Butyl-3-methylfurano[2,3-*d*]pyrimidin-2-one (3a). Treatment of 309 mg (1.5 mmol) of 2a in 10 mL of Et_3N/MeOH (3:7) with 10 mg of CuI at reflux for 4 h under N_2 was followed by evaporation of the solution. The dry residue was dissolved in 20 mL of $CHCl_3$, washed with 5% disodium EDTA/H_2O (2 × 20 mL), and 20 mL of H_2O, dried (Na_2SO_4), and evaporated. The residue was crystallized as described to give 285 mg (92%) of 3a that was identical with the 3a byproduct from the preceding experiment.

1-Methyl-5-[4-(*p*-toluyloxy)butynyl]uracil (2b). A 1.26-g (5 mmol) sample of 1 was suspended in 60 mL of deoxygenated Et_3N, and 1.88 g (10 mmol) of 4-(*p*-toluyloxy)butyne, 25 mg of $(Ph_3P)_2PdCl_2$, and 25 mg of CuI were added. The suspension was stirred at 50 °C for 1.5 h under N_2 followed by processing as described above for the conversion of 1 to 2a prior to chromatography. Evaporation of the organic phase to dryness gave a white solid that was triturated with Et_2O (2 × 50 mL) to give 1.32 g (85%) of TLC homogeneous 2b. This product was recrystallized from EtOH to give 2b: mp 147–150 °C; NMR δ 2.38 (s, 3, $ArCH_3$), 2.84 (t, 2, $C{\equiv}CCH_2$), 3.20 (s, 3, NCH_3), 4.31 (t, 2, CH_2OTol), 7.34 (d, 2, aromatic), 7.90 (m, 3, H6 and aromatic), 11.48 (br s, 1, NH). Anal. Calcd for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97. Found: C, 65.22; H, 5.24; N, 9.00.

5-(4-Hydroxybutynyl)-1-methyluracil (2c). A 3.12-g (10 mmol) sample of 2b was added to 300 mL of NH_3/MeOH (saturated at ~5 °C), and the suspension was stirred at 25 °C for 7 days. The resulting solution was evaporated, and the residue triturated with Et_2O (3 × 50 mL) to give 1.93 g (99%) of 2c as a colorless powder. This product was recrystallized from 95% EtOH to give 1.7 g (88%) of 2c: mp 204–208 °C; NMR (400 MHz) δ 2.50 (t, $C{\equiv}CCH_2$), 3.20 (s, 3, NCH_3), 3.52 (t, 2, CH_2OH), 4.85 (br s, 1, OH), 7.95 (s, 1, H6), 11.50 (br s, 1, NH). Anal. Calcd for $C_9H_{10}N_2O_3$: C, 55.67; H, 5.19; N, 14.43. Found: C, 55.44; H, 5.34; N, 14.46.

3′,5′-Di-*O*-acetyl-5-iodo-2′-deoxyuridine (4). A solution of 7.8 g (25 mmol) of 3′,5′-di-*O*-acetyl-2′-deoxyuridine[41] and 5.69 g (35 mmol) of ICl in 250 mL of CH_2Cl_2 was heated at reflux for 3 h and then cooled. Water (200 mL) was added, and the stirred mixture was treated with the *minimum* quantity of 2% $NaHSO_3$/H_2O necessary to decolorize the excess ICl. The organic phase was separated and washed with H_2O (2 × 200 mL), dried (Na_2SO_4), and evaporated. The resulting faintly yellow oil was dissolved in 100 mL of warm 98% EtOH, and this was cooled at −18 °C. The colorless crystals were filtered and washed with cold EtOH to give 9.65 g (88%) of 4 after drying: mp 157–159 °C (lit.[42] mp 158–160 °C); NMR δ 2.06 and 2.08 (2 s, 3 and 3, OAc's), 6.12 (t, 1, H1′), 8.03 (s, 1, H6), 11.72 (br s, 1, NH), plus sugar proton multiplets. Anal. Calcd for $C_{13}H_{15}IN_2O_7$: C, 35.63; H, 3.45; N, 6.39. Found: C, 35.80; H, 3.27; N, 6.32.

3′,5′-Di-*O*-acetyl-5-[2-(trimethylsilyl)ethynyl]-2′-deoxyuridine (5a). To 300 mL of deoxygenated Et_3N was added 3.51 g (8 mmol) of 4 followed by 1.57 g (16 mmol) of (trimethylsilyl)acetylene, 120 mg of $(Ph_3P)_2PdCl_2$, and 120 mg of CuI. The suspension was stirred at 50 °C for 3 h under N_2, evaporated thoroughly to a solid brown foam, and processed as in the conversion of 1 to 2a. Chromatography was effected by using a column of silica (150 g) packed in benzene with benzene/EtOAc (8:2) as the eluant. Appropriately pooled fractions were evaporated to give 2.62 g (80%) of 5a as a colorless solid foam. This was crystallized from EtOH to give 2.22 g (68%) of 5a as colorless plates: mp 178–179 °C; NMR δ 0.10 (s, 9, $Si(CH_3)_3$), 2.06 and 2.09 (2 s, 3 and 3, OAc's), 6.10 (t, 1, H1′), 7.94 (s, 1, H6), 11.68 (br s, 1, NH), plus sugar proton multiplets. Anal. Calcd for $C_{18}H_{24}N_2O_7Si$: C, 52.93; H, 5.92; N, 6.86. Found: C, 52.83; H, 6.02; N, 6.74.

3′,5′-Di-*O*-acetyl-5-(4-hydroxybutynyl)-2′-deoxyuridine (5b). A 2.63-g (6 mmol) sample of 4, 3.75 g (12 mmol) of 4-[(triphenylmethyl)oxy]butyne, 90 mg of $(Ph_3P)_2PdCl_2$, and 50 mg of CuI in 250 mL of deoxygenated Et_3N were stirred at 50 °C for 2.5 h under N_2. Solid disodium EDTA (1 g) was added followed by processing as in the conversion of 1 to 2a. The yellow solid foam was treated directly with 85% HOAc/H_2O at 50 °C for 1.5 h. Evaporation of this solution followed by addition and evaporation of toluene gave a solid foam that was chromatographed on a column of Mallinckrodt silica (150 g) with EtOAc as the eluant. Early fractions contained starting 4 (172 mg, 7%) followed by the deiodinated product, 3′,5′-di-*O*-acetyl-2′-deoxyuridine (198 mg, 11%). The title compound 5b (1.39 g, 61%) came next followed by 3-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-6-(2-hydroxyethyl)furano[2,3-*d*]pyrimidin-2-one (204 mg, 9%). The latter two compounds did not crystallize under several conditions. A small sample of 5b was deprotected by using NH_3/MeOH to give 5-(4-hydroxybutynyl)-2′-deoxyuridine (9m) (see Table II for characterization data). Similar deprotection of the final product eluted gave 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-6-(2-hydroxyethyl)furano[2,3-*d*]pyrimidin-2-one (140 mg, 88%). This compound was recrystallized from EtOH with diffusion of Et_2O to give a product: mp 162–164 °C; UV (pH 6 or 13) λ_{max} 328, 245, 225 nm (ε 6700, 11 500, 14 600), λ_{min} 267, 239 nm (ε 500, 11 100); NMR δ 2.20 (m, 2, H2′,2″), 2.78 (t, 2, $ArCH_2$), 3.70 (m, 4, H5′,5″ and CH_2OH), 3.90 (m, 1, H4′), 4.24 (m, 1, H3′), 6.16 (t, 1, H1′), 6.43 (s, 1, H5), 8.64 (s, 1, H4). Anal. Calcd for $C_{13}H_{16}N_2O_6$: C, 52.70; H, 5.44; N, 9.46. Found: C, 52.36; H, 5.65; N, 9.19.

5-Hexynyl-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (8c). To 160 mL of deoxygenated Et_3N was added 2.36 g (4 mmol) of 5-iodo-3′,5′-di-*o*-*p*-toluyl-2′-deoxyuridine[15] (7) followed by 656 mg (8 mmol) of hexyne, 60 mg of $(Ph_3P)_2PdCl_2$, and 60 mg of CuI. The resulting suspension was stirred at 50 °C for 4 h under N_2 and then thoroughly evaporated to dryness. The resulting yellow oil was dissolved in 200 mL of $CHCl_3$, washed with 5% disodium EDTA/H_2O (2 × 100 mL), and 100 mL of H_2O, dried (Na_2SO_4), evaporated, and redissolved in the minimum volume of hot $CHCl_3$. MeOH (5 volumes) was added, and the resulting precipitate was filtered to give 1.95 g (90%) of colorless and TLC-homogeneous 8c. This product was recrystallized from $CHCl_3$/MeOH to give 8c: mp 213–214 °C; UV (EtOH) λ_{max} 289, 238 nm (ε 9500, 34 100), λ_{min} 265 nm (ε 5900); NMR and Anal. (see Table I).

Compounds 8a–g,i,k,l,p were prepared from 7 and the respective terminal alkynes by the procedure for the conversion of

(40) Robins, M. J.; Mengel, R.; Jones, R. A.; Fouron, Y. *J. Am. Chem. Soc.* **1976**, *98*, 8204.
(41) Robins, M. J.; MacCoss, M.; Naik, S. R.; Ramani, G. *J. Am. Chem. Soc.* **1976**, *98*, 7381.
(42) Chang, P. K.; Welch, A. D. *J. Med. Chem.* **1963**, *6*, 428.

7 to 8c. Reactions with the more volatile alkynes (e.g., butyne) were effected by using a glass liner in a steel pressure bomb immersed in an oil bath at 55 °C. In cases where a colored reaction mixture resulted, the crude product was purified by passage through a short column of silica with MeOH/$CHCl_3$ (1:9) as the eluant before crystallization from MeOH/$CHCl_3$. See Scheme II and Table I for structures and data.

5-Ethynyl-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (8h). To 50 mL of anhydrous acetonitrile were added 560 mg (1 mmol) of 8g, 116 mg (2 mmol) of KF, and 420 mg (2 mmol) of Et_4NBr, and the stirred suspension was heated at reflux for 3 h. The resulting clear solution was evaporated, and the residue was dissolved in 80 mL of $CHCl_3$. This solution was washed with H_2O (3 × 80 mL), dried (Na_2SO_4), and evaporated. The resulting white solid was recrystallized from MeOH to give 410 mg (84%) of 8h that was identical with an authentic sample.[14]

5-(3-Hydroxypropynyl)-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (8j). A solution of 2.71 g (4.5 mmol) of 8i in 30 mL of CH_2Cl_2/MeOH/CF_3CO_2H (15:10:5) was stirred at 25 °C for 30 min and then was evaporated. Several portions of MeOH were added and evaporated, and the residue was crystallized from MeOH/$CHCl_3$ to give 2.11 g (90%) of 8j with the properties listed in Table I.

5-(4-Hydroxybutynyl)-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (8m). Treatment of 617 mg (1 mmol) of 8k for 1.5 h under the conditions described above for 8i → 8j gave a residue that was crystallized from 95% EtOH to give 479 mg (90%) of 8m with the properties listed in Table I.

5-[4-(*p*-Toluenesulfonyloxy)butynyl]-3′,5′-di-*O*-*p*-toluyl-2′-deoxyuridine (8n). A solution of 293 mg (0.55 mmol) of 8m and 286 mg (1.5 mmol) of tosyl chloride in 20 mL of pyridine was stirred at 25 °C for 18 h and then evaporated. The resulting yellow oil was triturated with hexane (2 × 40 mL), and then 15 mL of MeOH was added. The suspension was cooled at −18 °C for several hours and filtered, and the collected solid was recrystallized from MeOH/$CHCl_3$ to give 246 mg (65%) of 8n with the properties listed in Table I.

2′,3′,5′-Tri-*O*-*p*-toluyl-5-[4-(*p*-toluyloxy)butynyl]uridine. Treatment of 4.35 g (6 mmol) of 5-iodo-2′,3′,5′-tri-*O*-*p*-toluyluridine[15] with 1.5 g (8 mmol) of 4-(*p*-toluyloxy)butyne under the general coupling conditions described for the conversion of 7 to 8c gave 4.33 g (92%) of the crystalline title compound: mp 215–218 °C; NMR ($CDCl_3$) δ 2.40 (br s, 9, $ArCH_3$'s), 2.68 (t, 2, $C{\equiv}CCH_2$), 4.25 (t, 2, CH_2OTol), 6.30 (d, 1, H1′), 7.10–8.05 (m, 13, H6 and aromatic), 8.63 (br s, 1, NH), plus sugar proton multiplets. Anal. Calcd for $C_{45}H_{40}N_2O_{11}$: C, 68.87; H, 5.14; N, 3.57. Found: C, 69.05; H, 5.25; N, 3.79.

5-(4-Hydroxybutynyl)uridine. To 40 mL of 0.1 N sodium methoxide in anhydrous methanol was added 3.92 g (5 mmol) of 2′,3′,5′-tri-*O*-*p*-toluyl-5-[4-(*p*-toluyloxy)butynyl]uridine, and the mixture was stirred at 25 °C for 6 h. TLC indicated that deprotection was complete. The solution was carefully neutralized by addition of Dowex 50-X8 (H^+) resin until moistened pH paper indicated pH ~6. The mixture was filtered, and the resin was washed with MeOH. The combined filtrate was evaporated, and the colorless residue was triturated with Et_2O (3 × 50 mL). The resulting TLC-homogeneous white powder (1.53 g, 98%) was crystallized from 95% EtOH with diffusion of Et_2O[40] to give 1.26 g (81%) of the title compound: mp 213–215 °C; UV (pH 6) λ_{max} 292, 232 nm (ε 12 500, 12 300), λ_{min} 257 nm (ε 3300); UV (pH 13) λ_{max} 287, 230 nm (ε 9900, 14 500), λ_{min} 261 nm (ε 4700); NMR δ 2.50 (m, 2, $C{\equiv}CCH_2$ and solvent), 3.40–4.10 (m, 7, CH_2OH and sugar protons), 4.80–5.40 (m, 4, OH's), 5.76 (d, 1, H1′), 8.18 (s, 1, H6), 11.60 (br s, 1, NH). Anal. Calcd for $C_{13}H_{16}N_2O_7$: C, 50.00; H, 5.16; N, 8.97. Found: C, 49.83; H, 5.18; N, 8.76.

Compounds 9a–f,j,m,q were prepared by deprotection of their corresponding *p*-toluyl ester precursors 8a–f,j(l or m),p by using 0.1 or 0.2 N NaOMe/MeOH as described in the above procedure for 5-(4-hydroxybutynyl)uridine. An Et_2O/hexane mixture was used in place of Et_2O for trituration of the more lipophilic compounds. Yields of the TLC-homogeneous triturated products usually ranged from 90% to 98%. These solids were recrystallized from MeOH or EtOH with diffusion of Et_2O[40] to give samples with the properties listed in Table II.

5-Ethynyl-2′-deoxyuridine (9h) was prepared directly from the protected 5-[(trimethylsilyl)ethynyl] precursor (8g) by using 0.2 N NaOMe/MeOH according to the above general procedure. TLC homogeneous 9h was obtained quantitatively and could be recrystallized as described to give a product identical with a known sample.[14]

5-[4-(*p*-Toluenesulfonyloxy)butynyl]-2′-deoxyuridine (9n). A 465-mg (0.68 mmol) sample of 8n was deprotected by using 10 mL of 0.1 N NaOMe/MeOH at 25 °C for 5 h. Processing by the above general procedure gave a colorless solid that was triturated with hexane (3 × 40 mL) and then extracted into Et_2O. This Et_2O solution was allowed to evaporate to a small volume, and 190 mg (60%) of 9n crystallized as its monohydrate with the properties listed in Table II.

5-(But-3-en-1-ynyl)-2′-deoxyuridine (9o). To 10 mL of anhydrous acetonitrile were added 206 mg (0.3 mmol) of 8n and 146 mg (1.3 mmol) of potassium *tert*-butoxide. The suspension was stirred vigorously and subjected to ultrasound (sonication in a commercial cleaning bath) for 20 min. TLC (MeOH/$CHCl_3$, 1:9) indicated the complete disappearance of starting 8n. Anhydrous MeOH (10 mL) was added, and the mixture was stirred at 25 °C for 18 h. Careful neutralization with Dowex 50-X8 (H^+) resin was followed by filtration, washing of the resin with MeOH, and evaporation of the combined filtrate to a small volume. This was chromatographed on a 20 × 20 cm Merck preparative TLC silica plate with MeOH/$CHCl_3$ (1:9). The major UV quenching band was eluted and crystallized with difficulty from EtOH/Et_2O (1:1) with diffusion of Et_2O[40] to give 34 mg (39%) of 9o as its hemihydrate with the properties listed in Table II.

1-[(2-Hydroxyethoxy)methyl]-(*E*)-5-(oct-1-en-3-ynyl)uracil (11). To 40 mL of deoxygenated Et_3N were added 333 mg (1 mmol) of 1-[(2-acetoxyethoxy)methyl]-(*E*)-5-(2-bromovinyl)uracil[28] (10), 250 mg (3 mmol) of hexyne, 15 mg of $(Ph_3P)_2PdCl_2$, and 15 mg of CuI. The mixture was stirred at 50 °C for 1.5 h under N_2 and then processed as described for the conversion of 7 to 8c with passage through a column (50 g) of silica. The resulting yellow oil (317 mg, 95%) had NMR spectral data in harmony with the expected coupling product. It did not crystallize under several conditions. A 287-mg (0.86 mmol) sample was deprotected with 0.05 N NaOMe/MeOH by using the general procedure to give 191 mg (76%) of 11 as a yellow solid. Recrystallization of this product from EtOH/H_2O gave pale yellow plates of 11: mp 118–121 °C; UV (EtOH) λ_{max} 267, 240, 229 nm (ε 11 100, 13 400, 14 900), λ_{min} 250, 238 nm (ε 9700, 13 000); NMR (400 MHz) δ 0.92 (t, 3, CH_2CH_3), 1.45 (m, 4, $CH_2CH_2CH_3$), 2.39 (m, 2, $C{\equiv}CCH_2$), 3.54 (m, 4, OCH_2CH_2OH), 4.70 (t, 1, OH), 5.14 (s, 2, NCH_2O), 6.54 (d, J = 16 Hz, 1, vinylic H1), 6.60 (dt, J_{vic} = 16 Hz, $^5J_{3-5}$ = 2 Hz, 1, vinylic H2), 8.00 (s, 1, H6), 11.50 (br s, 1, NH). Anal. Calcd for $C_{15}H_{20}N_2O_4$: C, 61.63; H, 6.90; N, 9.58. Found: C, 61.47; H, 7.16; N 9.33.

5-Ethyl-2′-deoxyuridine (12). To a solution of 201 mg (0.8 mmol) of 9h in 10 mL of EtOH was added 100 mg of 5% Pd/C catalyst, and the suspension was hydrogenated at 1 atm for 1 h. TLC indicated that reaction was complete. Catalyst was removed by filtration and washed with 20 mL of EtOH. The combined filtrate was evaporated to give 198 mg (97%) of 12 as a colorless crystalline solid. This was recrystallized from EtOH with diffusion of Et_2O[40] to give 12: mp 151–152 °C (lit.[29a,30a] mp 152–153 °C); UV (pH 6) λ_{max} 268 nm (ε 10 400), λ_{min} 236 nm (ε 3300); NMR δ 1.04 (t, 3, CH_2CH_3), 2.06–2.38 (m, 4, H2′, 2″ and CH_2CH_3), 3.61 (m, 2, H5′, 5″), 3.78 (m, 1, H4′), 4.26 (m, 1, H3′), 6.20 (t, 1, H1′), 7.69 (s, 1, H6). Anal. Calcd for $C_{11}H_{16}N_2O_5$: C, 51.56; H, 6.29; N, 10.93. Found: C, 51.25; H, 6.31; N, 10.84.

5-*n*-Hexyl-2′-deoxyuridine (13). Hydrogenation of 154 mg (0.5 mmol) of 9c (by the above procedure used for 9h → 12) followed by recrystallization of the product from acetone/hexane gave 144 mg (92%) of 13: mp 104–106 °C (lit.[30a] mp 101 °C); UV (pH 6) λ_{max} 269 nm (ε 9300), λ_{min} 236 nm (ε 2400); NMR (400 MHz) δ 0.86–2.20 (5 m, 13, hexyl chain), 3.57 (m, 2, H5′, 5″), 3.77 (m, 1, H4′), 4.23 (m, 1, H3′), 6.19 (t, 1, H1′), 7.70 (s, 1, H6). Anal. Calcd for $C_{15}H_{24}N_2O_5$: C, 57.68; H, 7.74; N, 8.97. Found: C, 57.38; H, 7.70; N, 8.81.

3′,5′-Di-*O*-acetyl-5-[2-(trimethylsilyl)ethyl]-2′-deoxyuridine (6a). Hydrogenation of 204 mg (0.5 mmol) of 5a (by the procedure used for 9h → 12) gave two products that were separated by preparative HPLC with 42% CH_3CN/H_2O at a flow rate of 3 mL/min. The first product eluted (38 mg, 22%) was identified by its NMR spectrum as 3′,5′-di-*O*-acetyl-5-ethyl-2′-

deoxyuridine (6b). Deprotection of this product gave 12 which was identified by comparison with the sample of 12 prepared above.

Elution of the preparative HPLC column with 42–50% CH_3CN/H_2O gave 137 mg (67%) of the major product (6a) that was recrystallized from EtOH to give 6a: mp 132–134 °C; NMR δ 0.00 (s, 9, $Si(CH_3)_3$), 0.60 (m, 2, CH_2Si), 2.08 (br s, 6, OAc's), 2.30 (m, 4, H2′,2″ and CH_2CH_2Si), 6.18 (t, 1, H1′), 7.38 (s, 1, H6), 11.32 (br s, 1, NH), plus sugar proton multiplets. Anal. Calcd for $C_{18}H_{28}N_2O_7Si$: C, 52.41; H, 6.84; N, 6.79. Found: C, 52.31; H, 6.74; N, 6.69.

(*Z*)-5-Hexenyl-2′-deoxyuridine (14). To a solution of 308 mg (1 mmol) of 9c in 100 mL of acetone was added 1 mL of freshly distilled quinoline and 0.5 g of Lindlar catalyst. The mixture was hydrogenated at 1 atm for 80 min at 25 °C. Analytical HPLC (27% CH_3CN/H_2O, flow rate 1 mL/min) showed the virtual absence of starting 9c. The suspension was filtered and the filter cake washed with 100 mL of acetone. The combined filtrate was evaporated, and the residual yellow oil was chromatographed on a short (10 g) column of silica with $CHCl_3$ as the eluant to remove traces of quinoline. Nucleoside products were eluted with $MeOH/CHCl_3$ (1:9), and appropriate fractions were pooled and evaporated. The colorless solid residue was dissolved in 2 mL of EtOH, and 1-mL portions were subjected to preparative HPLC (22% CH_3CN/H_2O followed by 22–30% CH_3CN/H_2O at a flow rate of 3 mL/min). Appropriate 22% acetonitrile fractions were pooled and evaporated to give 258 mg (83%) of 14 as a crystalline white solid. This was recrystallized from $CHCl_3$ to give 14: mp 137–139 °C; UV (pH 6) λ_{max} 282, 229 nm (ϵ 7700, 15 100), λ_{min} 258 nm (ϵ 4800); UV (pH 13) λ_{max} 278, 228 nm (ϵ 6300, 16 900) λ_{min} 265 nm (ϵ 5600); NMR (400 MHz) δ 0.90 (m, 3, CH_2CH_3), 1.30 (m, 4, $CH_2CH_2CH_3$), 2.10 (m, 4, H2′,2″ and $CH{=}CHCH_2$), 3.54 (m, 2, H5′,5″) 3.83 (m, 1, H4′), 4.26 (m, 1, H3′), 5.60 (d t, 1, $CH{=}CHC_4H_9$), 6.03 (d, J_{vin} = 11.5 Hz, 1, $CH{=}CHC_4H_9$), 6.23 (t, 1, H1′), 7.80 (s, 1, H6). Anal. Calcd for $C_{15}H_{22}N_2O_5$: C, 58.05; H, 7.15; N, 9.03. Found: C, 57.78; H, 7.25; N, 8.77.

Appropriately pooled 22–30% acetonitrile fractions were evaporated to give 31 mg (10%) of 13, identical with the sample of 13 prepared above.

(*E*)-5-(2-Bromovinyl)-2′-deoxyuridine (15). To a solution of 816 mg (2 mmol) of 5a in 50 mL of EtOAc were added 0.5 mL of freshly distilled quinoline and 1 g of Lindlar catalyst. The mixture was hydrogenated at 1 atm for 5 h at 25 °C. The catalyst was filtered and washed with 50 mL of EtOAc. The combined filtrate was washed with cold 1 N HCl/H_2O (2 × 30 mL) and H_2O (2 × 30 mL) and evaporated to give 780 mg (95%) of a colorless solid foam. The ^1H NMR spectrum of this crude product revealed the presence of traces of starting 5a and the fully saturated 6a along with 3′,5′-di-*O*-acetyl-(*Z*)-5-[2-(trimethylsilyl)ethenyl]-2′-deoxyuridine (6c) as the highly predominant component: NMR (400 MHz) δ 0.06 (s, 9, $Si(CH_3)_3$), 2.00 and 2.06 (2 s, 3 and 3, OAc's), 5.77 (d, J_{vin} = 15 Hz, 1, vinylic H2), 6.13 (t, 1, H1′), 6.82 (d, J_{vin} = 15 Hz, 1, vinylic H1), 7.40 (s, 1, H6), plus sugar proton multipleta.

A stirred solution of 369 mg (0.9 mmol) of crude 6c in 20 mL of CS_2 was cooled to −100 °C and treated dropwise over 20 min with a cold (−80 °C) solution of 144 mg (0.9 mmol) of Br_2 in 5 mL of CS_2. The mixture was stirred for an additional 15 min at −100 °C and then allowed to warm to room temperature. Acetonitrile (10 mL) was added, and the resulting mixture was evaporated to dryness and treated directly with 15 mL of NH_3/MeOH (saturated at ~5 °C) for 18 h at 25 °C. This solution was evaporated, and the resulting oil was purified by preparative TLC with $MeOH/CHCl_3$ (1:9). The major band was eluted, and the colorless residue was further purified by preparative HPLC (14% CH_3CN/H_2O, flow rate 2 mL/min) to give 120 mg (40%) of 15 that was recrystallized and found to be identical with a purified authentic sample of 15.[20]

5-(4-Hydroxybutanoyl)-1-methyluracil (17). A stirred solution of 1.55 g (8 mmol) of 2c in 160 mL of hot H_2O was cooled to 50 °C, and 160 mg of $HgSO_4$ was added. The mixture was stirred at 50 °C for 2 h, cooled to 25 °C, and saturated with H_2S. This suspension was filtered by using a Celite pad, and the filter cake was washed with 50 mL of MeOH. The combined filtrate was evaporated, 50 mL of Me_2CO/H_2O (1:1) was added and evaporated, and 50 mL of Me_2CO was added and evaporated. The residual faintly yellow solid was crystallized from Me_2CO to give 878 mg (52%) of 17. An additional 450 mg (26%) of 17 was recovered by evaporation of the mother liquor and preparative TLC of the residue with $MeOH/CHCl_3$ (1:19) with multiple developments. The faster migrating band was eluted, and the product crystallized from Me_2CO to give a total yield of 1.328 g (78%) of 17: mp 170–172 °C; NMR (200 MHz) δ 1.65 (m, 2, CH_2CH_2OH), 2.91 (t, 2, $COCH_2$), 3.26 (m, 5, NCH_3 and CH_2OH), 4.44 (m, 1, OH), 8.43 (s, 1, H6), 11.50 (br s, 1, NH). Anal. Calcd for $C_9H_{12}N_2O_4$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.88; H, 5.67; N, 13.23.

The slower migrating and highly fluorescent band was eluted from the TLC plate to give 76 mg (5%) of a colorless solid that had spectral properties consistent with 6-(2-hydroxyethyl)-3-methylfurano[2,3-*d*]pyrimidin-2-one (3c): mp 240–245 °C dec; UV (pH 6 or pH 13) λ_{max} 324, 242, 225 nm (ϵ 5100, 11 800, 13 400), λ_{min} 265, 236 nm (ϵ 900, 11 500); NMR δ 2.77 (t, 2, CH_2CH_2OH), 3.50 (s, 3, NCH_3), 3.70 (m, 2, CH_2OH), 4.78 (t, 1, OH), 6.42 (s, 1, H5), 8.44 (s, 1, H4).

5-(4-Hydroxybutanoyl)-2′-deoxyuridine (18). To a solution of 148 mg (0.5 mmol) of 9m in 5 mL of H_2O was added 10 mg of $HgSO_4$, and stirring was continued at 25 °C for 2 h. The solution was then saturated with H_2S, and the resulting suspension was applied directly to a preparative TLC plate and multiply developed by using the upper phase of *n*-PrOH/H_2O/EtOAc (1:2:4). The major UV quenching band was eluted, and evaporation of solvent gave 101 mg (64%) of 18. This was crystallized with difficulty from CH_3CN/H_2O to give 48 mg (30%) of 18·0.5H_2O: mp 89–91 °C; UV (pH 6) λ_{max} 284, 229 nm (ϵ 12 600, 9800) λ_{min} 249 nm (ϵ 2400); UV (pH 13) λ_{max} 284, 230 (sh) nm (ϵ 9400, 11 000) λ_{min} 258 nm (ϵ 2400); NMR (200 MHz) δ 1.68 (m, 2, CH_2CH_2OH), 2.20 (m, 2, H2′,2″), 2.95 (t, 2, $COCH_2$), 3.41 (m, 2, CH_2OH), 3.59 (m, 2, H5′,5″), 3.86 (m, 1, H4′), 4.24 (m, 1, H3′), 6.13 (t, 1, H1′), 8.66 (s, 1, H6). Anal. Calc for $C_{13}H_{18}N_2O_7$·0.5H_2O: C, 48.30; H, 5.92; N, 8.66. Found: C, 48.40; H, 5.80; N, 8.38.

5-(4-Hydroxybutanoyl)uridine (19). A 312-mg (1 mmol) sample of 5-(4-hydroxybutynyl)uridine was dissolved in 10 mL of H_2O, treated with 20 mg of $HgSO_4$, and processed as described for the above conversion of 9m to 18. The 220 mg (67%) of crude 19 obtained from the TLC plate was recrystallized from $MeOH/H_2O$ to give 19: mp 170–173 °C; UV (pH 6) λ_{max} 284, 230 nm (ϵ 13 500, 11 300) λ_{min} 251 nm (ϵ 3100); UV (pH 13) λ_{max} 286, 235 (sh) nm (ϵ 10 200, 12 300) λ_{min} 260 nm (ϵ 4300); NMR (200 MHz) δ 1.65 (m, 2, CH_2CH_2OH), 2.90 (t, 2, $COCH_2$), 3.39 (m, 2, CH_2OH), 3.60 (m, 2, H5′,5″), 3.90 (m, 2, H3′, H4′), 4.10 (m, 1, H2′), 5.81 (d, 1, H1′), 8.74 (s, 1, H6). Anal. Calcd for $C_{13}H_{18}N_2O_8$: C, 47.27; H, 5.49; N, 8.48. Found: C, 47.08; H, 5.52; N, 8.18.

3′,5′-Di-*O*-acetyl-5-(4-hydroxybutanoyl)-2′-deoxyuridine (20). A stirred suspension of 571 mg (1.5 mmol) of 5b in 10 mL of H_2O was warmed to effect solution and then cooled to 25 °C. Stirring was continued for 2 h after addition of 30 mg of $HgSO_4$. The solution was saturated with H_2S, and the resulting suspension was evaporated to dryness. EtOAc (~5 mL) was added, and the slurry was applied to a column (50 g) of silica. The column was developed with EtOAc. Appropriate fractions were pooled and evaporated to give 351 mg (59%) of 20 as a white solid foam. This was crystallized from EtOH with diffusion of Et_2O[40] to give colorless needles of 20: mp 146–148 °C; NMR δ 1.68 (m, 2, CH_2CH_2OH), 2.06 and 2.09 (2 s, 3 and 3, OAc's), 2.94 (t, 2, $COCH_2$), 3.38 (m, 2, CH_2OH), 6.12 (t, 1, H1′), 8.36 (s, 1, H6), 11.68 (br s, 1, NH), plus sugar proton multiplets. Anal. Calcd for $C_{17}H_{22}N_2O_9$: C, 51.26; H, 5.57; N, 7.03. Found: C, 50.95; H, 5.51; N, 6.84.

6-*n*-Butyl-3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-furano[2,3-*d*]pyrimidin-2-one (16). Method A. To a solution of 154 mg (0.5 mmol) of 9c in 10 mL of Et_3N/MeOH (3:7) was added 10 mg of CuI, and the solution was heated at reflux for 3 h. Volatile materials were evaporated, and the residue was taken up in 20 mL of $CHCl_3$ and washed with 2% disodium EDTA/H_2O (2 × 10 mL) and 10 mL of H_2O. The combined aqueous layers were extractd with $CHCl_3$ (2 × 250 mL). The combined organic layers were dried (Na_2SO_4) and evaporated to give a white solid. This product was crystallized from EtOH with diffusion of Et_2O[40] to give 126 mg (82%) of 16 as colorless needles: mp 150–152 °C; UV (pH 6) λ_{max} 327, 245, 224 nm (ϵ 6600, 11 700, 15 400), λ_{min} 268, 239 nm (ϵ 1300, 11 400); NMR (200 MHz) δ 0.92 (t, 3, CH_2CH_3),

1.30–1.70 (m, 4, $CH_2CH_2CH_3$), 2.20 (m, 2, H2′,2″), 2.66 (t, 2, $ArCH_2$), 3.91 (m, 2, H5′,5″), 4.24 (m, 1, H3′), 6.18 (t, 1, H1′), 6.44 (s, 1, H5), 8.68 (s, 1, H4). Anal. Calcd for $C_{15}H_{20}N_2O_5$: C, 58.43; H, 6.54; N, 9.09. Found: C, 58.37; H, 6.43; N, 8.91.

Method B. To a solution of 308 mg (1 mmol) of 9c in 4 mL of dioxane/H_2O (1:1) was added 10 mg of $HgSO_4$, and the mixture was stirred at 25 °C for 48 h. The major, highly fluorescent product (110 mg, 36%) was isolated by preparative TLC and shown to be 16 (mp 150–152 °C), identical with the product of method A.

Acknowledgment. We thank the National Cancer Institute of Canada, the Natural Sciences and Engineering Research Council of Canada (Grant No. A5890), and The University of Alberta for generous support. M.J.R. thanks Professor A. D. Broom for facilities and hospitality provided during a visiting professorship at the University of Utah, 1981–1982.

Registry No. 1, 45774-47-8; 2a, 77875-79-7; 2b, 77875-82-2; 2c, 85267-59-0; 3a, 77875-80-0; 3c, 85267-72-7; 4, 1956-30-5; 5a, 85267-60-3; 5b, 85267-61-4; 6a, 85282-15-1; 6b, 59864-07-2; 6c, 85267-70-5; 7, 31356-86-2; 8a, 77875-86-6; 8b, 77875-87-7; 8c, 85267-63-6; 8d, 77875-89-9; 8e, 77875-90-2; 8f, 77875-92-4; 8g, 77875-91-3; 8h, 69075-43-0; 8i, 77875-93-5; 8j, 85267-64-7; 8k, 77875-94-6; 8l, 77882-22-5; 8m, 85267-65-8; 8n, 85267-66-9; 8p, 77875-95-7; 9a, 77875-96-8; 9b, 77887-18-4; 9c, 77875-97-9; 9d, 77875-98-0; 9e, 77887-19-5; 9f, 77887-20-8; 9h, 61135-33-9; 9j, 77875-99-1; 9m, 77876-00-7; 9n, 84559-05-7; 9o, 84582-78-5; 9q, 77876-01-8; 10, 85267-69-2; 11, 85267-68-1; 12, 15176-29-1; 13, 57741-93-2; 14, 84621-32-9; 15, 69304-47-8; 16, 85267-76-1; 17, 85267-71-6; 18, 85267-73-8; 19, 85267-74-9; 20, 85267-75-0; $(Ph_3P)_2PdCl_2$, 13965-03-2; CuI, 7681-65-4; 4-(*p*-toluyloxy)butyne, 77875-81-1; 3-butynol, 2028-63-9; 5-(*p*-toluyloxy)pentyne, 77875-85-5; 4-pentynol, 2117-11-5; hexyne, 693-02-7; 3′,5′-di-*O*-acetyl-2′-deoxyuridine, 13030-62-1; (trimethylsilyl)acetylene, 1066-54-2; 4-[(triphenylmethyl)oxy]butyne, 75014-48-1; 3-(3,5-di-*O*-acetyl-2-deoxy-β-D-*erythro*-pentofuranosyl)-6-(2-hydroxyethyl)furano[2,3-*d*]pyrimidin-2-one, 85267-77-2; 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-6-(2-hydroxyethyl)furano[2,3-*d*]pyrimidin-2-one, 85267-62-5; 1-butyne, 107-00-6; 1-pentyne, 627-19-0; 1-heptyne, 628-71-7; 3,3-dimethyl-1-butyne, 917-92-0; benzeneethyne, 536-74-3; trimethylsilylethyne, 1066-54-2; 3-(tetrahydropyranyloxy)-1-propyne, 6089-04-9; 4-(tetrahydropyranyloxy)-1-butyne, 40365-61-5; 2′,3′,5′-tri-*O*-*p*-toluyl-5-[4-(*p*-toluyloxy)butynyl]uridine, 77875-84-4; 5-iodo-2′,3′,5′-tri-*O*-*p*-toluyluridine, 77875-83-3; 5-(4-hydroxybutynyl)uridine, 85267-67-0.

Synthesis and Photophysical Properties of Some *endo*-6-Substituted Norcamphors and Pentacyclo[5.4.0.0^{2,6}.0^{3,10}.0^{5,9}]undecan-8-ones

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The reactivity of the π* region of photoexcited carbonyl groups was studied by monitoring the fluorescence of a series of substituted norcamphors and pentacycloundecanones. These substrates contained various probes so positioned as to be able to interact directly with the π*-orbital system of the carbonyl group but not the n-orbital system. No significant perturbation of carbonyl fluorescence was caused by weak electron donors (Cl, OR, SR, and OTs), proton sources (OH, CO_2H), electron sinks (C≡N), and abstractable hydrogen atoms (CH_3, CH_2X). By contrast, the dimethylamino group was able to completely quench fluorescence, presumably via an electron-transfer mechanism. These results support the concept of reduced electrophilicity of the π* region of an excited carbonyl group.

With few exceptions the photochemistry of saturated carbonyl compounds has been rationalized in terms of the properties of the electron-deficient (in plane) molecular orbital system that results from promotion of a n electron to the π* orbital. The diminished electron density in the (delocalized) n-orbital system gives rise to its well-known electrophilic properties, e.g., hydrogen abstractions, additions to electron-rich olefins, and type I and II cleavages. On the other hand, relatively little chemistry has been associated with the electron-rich region above and below the plane of the carbonyl group.[2-4] In fact, much of the experimental work does not differentiate between reactions occurring in the two orthogonal planes since no fixed geometrical relationships were imposed on the reacting centers. We have initiated a program whose objectives are to design experiments that will delineate the stereoelectronic requirements of common photochemical reactions. Our approach to these objectives is to examine the properties of rigidly oriented systems that contain various internal probes positioned to interact *only* with the electronic system above (or below) the plane of the carbonyl group.

In this report we detail some studies that utilize the fluorescent properties of alkanones to monitor the reactivity of various probes with π* systems of excited carbonyl groups. The two molecular systems evaluated were a series of 6-*endo*-norcamphors (1a–f and 2) and some pentacycloundecanones (3a–h).

Syntheses: Norcamphors. The key starting material for the syntheses of 1a–f was the keto acid 2 first reported by Beckmann and Geiger.[5] This compound was converted in one step to the methyl ester dimethyl ketal 4 by heating

(1) On leave from Helwan University, Giza, Egypt.
(2) For references and a summary, see: Turro, N. J.; Dalton, J. C.; Farrington, G.; Niemcyzk, M.; Pond, D. M. *J. Am. Chem. Soc.* 1970, *92*, 6978.
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(5) Beckmann, S.; Geiger, H. *Chem. Ber.* 1961, *94*, 48.

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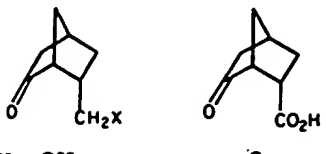
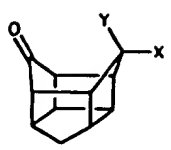
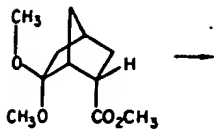


1a, X = OH
1b, X = Cl
1c, X = $OSO_2C_7H_7$
1d, X = CN
1e, X = $SCH_2C_6H_5$
1f, X = $N(CO)_2C_6H_4$

2



3a, X,Y = H
3b, X,Y = OCH_2CH_2O
3c, X,Y = SCH_2CH_2S
3d, X,Y = O
3e, X = C_6H_5; Y = H
3f, X,Y = OCH_3
3g, X = H; Y = $N(CH_3)_2$
3h, X = H; Y = CH_3



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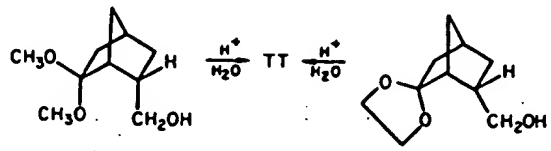
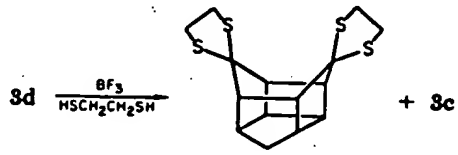


5 6

with trimethyl orthoformate in the presence of acid. Reduction of the latter compound by lithium aluminum hydride gave the ketal 5, which was hydrolyzed to give 1a. The stereochemistry of 1a was confirmed by an alternate synthesis from 6 a molecule whose structure had been proven earlier.[6]

The chloride 1b was prepared from 1a by using thionyl chloride in pyridine, and the tosylate 1c by Tipson's procedure.[7] The other three compounds were prepared by displacement reactions using cyanide ion,[8] benzylmercaptide ion, and phthalimido ion.[9]

Pentacycloundecanones. Compounds 3a,[10] 3b,[11] and 3d[12] were prepared by literature procedures. Compound 3c was not readily obtained on treatment of diketone 3d with $HSCH_2CH_2SH{\cdot}BF_3$.[13] Instead, we obtained a mixture of products, one of which had physical properties consistent with those expected for a bis(thioketal), i.e., 7.



3d + 3c

7

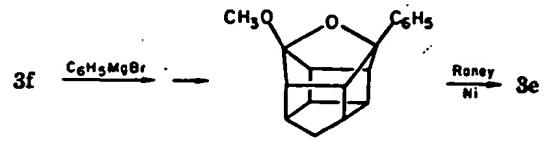
A second compound, mp 117 °C, was also isolated from this reaction. Its properties were those anticipated for the monothioketal 3c except that our melting point differed from that reported (lit.[13] mp 85–86 °C). We also obtained the product with mp 117 °C from the reaction of 3d with ethanedithiol catalyzed with *p*-toluenesulfonic acid. It is possible that the two compounds are polymorphs since the only discrepancy appears to be in the melting points.

Table I. Absorption and Relative Fluorescence Data of Norcamphors

compd	concn, M	λ_{max} (±2 nm)	ϵ_{max} (±5%)	V_f[a,b] (±5 nm)	ϕ_f^{rel}[c] (±15%)
norcamphor	0.040	291	25	404	1.0
1a	0.040	293	36	410	0.80
1b	0.040	292	38	413	0.84
1c	0.040	294	36	410	1.2
1d	0.042	290	40	395	1.1
1e	0.0059	289	87	405	1.3
1f	0.00046	292	1879	383	
2	0.040	292	45	404	0.92

[a] Acetonitrile solutions. [b] Excitation at 310 or 313 nm. [c] Ratios corrected for unequal light absorption or determined at equal absorbance; cf: Parker, C. A. "Photoluminescence of Solutions"; American Elsevier: New York, 1968; pp 15–21.

The phenyl ketone 3e was to be prepared by reacting the mono ketal 3f[14] with phenylmagnesium bromide followed by hydrogenolysis. The expected ketal carbinol was not produced in the Grignard synthesis. Instead, the product (NH_4Cl workup) displayed only one methoxyl group (NMR) and no hydroxyl group (IR). We conclude that the reaction product was 8. In any event, when



8

compound 8 was subjected to hydrogenolysis using Raney nickel, a phenyl ketone was produced.[15] The stereochemistry of the phenyl ring was deduced from an analysis of the NMR spectrum in the presence and absence of the shift reagent Eu(fod)$_3$.[16] Before addition of the shift reagent we had assigned a sharp singlet (width at half-height $\simeq$ 2.5 Hz) at δ 3.1 to the benzylic proton. It was concluded that this proton must have the syn configuration since significant coupling with the protons on C_1 and C_{10} would be expected for the anti configuration (dihedral angles ~40°) but not the syn configuration (dihedral angles ~80°). This assignment was confirmed when it was found that the peak at δ 3.1 and two other protons were the most strongly shifted protons on addition of Eu(fod)$_3$.[17]

The method of Borch and Hassid[18] was used to prepare the dimethylamino ketone 3g. The configuration was assigned by analysis of spectroscopic data, which indicated transannular interaction between the carbonyl group and the amine. For example, whereas most of the compounds in this series display carbonyl absorptions between 5.8 and

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(17) Raber, D. J.; Janks, C. M.; Johnston, M. D., Jr.; Raber, N. K. *J. Am. Chem. Soc.* 1980, *102*, 6591. The two bridgehead protons at C_7 and C_9 and the syn-H_{11} proton are similarly disposed in the complex.
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PALLADIUM CATALYSED C-8 ALLYLATION AND VINYLATION OF ADENOSINE, 2'-DEOXYADENOSINE AND 2',3'-DIDEOXYADENOSINE NUCLEOSIDES

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Abstract:
Using a coupling reaction between 8-iodo derivatives of O-TBDMS protected adenosine, 2'-deoxyadenosine, 2',3'-dideoxyadenosine and either vinyltributyltin or allyltributyltin with $Pd(PPh_3)_4$ catalysis, the corresponding 8-substituted nucleosides were obtained in excellent yields.

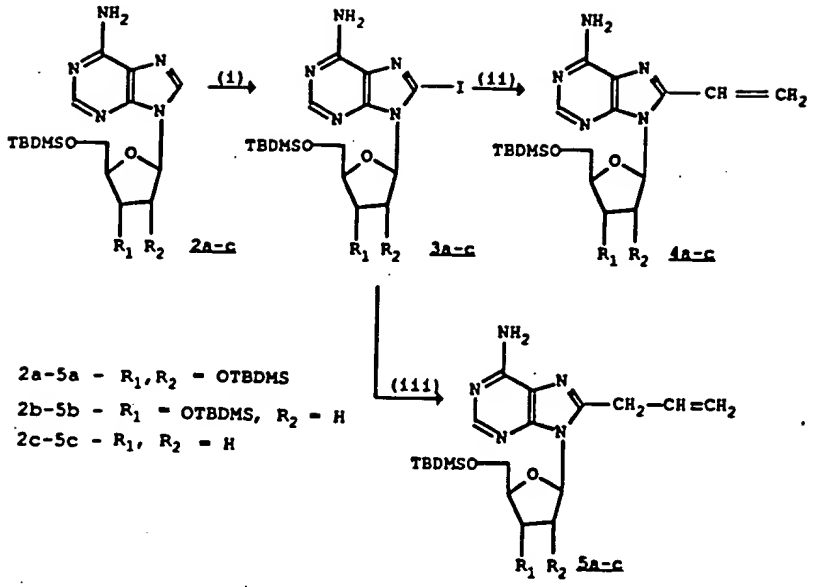
Modified 2-'deoxy and 2',3'-dideoxynucleosides are of interest due to their remarkable biological activity, particularly within the context of anti-viral therapeutic agents[1-3]. Such modified nucleoside analogues can be used for example, in the design of 'antisense' polynucleotides[4,5],sequence specific DNA cleaving agents[6]and in sequencing DNA[7,8].

Several methods have been reported for the functionalization[9] of the C-8 position of purine nucleosides. Direct bromination (in a pH controlled buffer) at the C-8 position[10a,b] and subsequent nucleophilic displacement was one of the earliest approaches. Synthetically more satisfactory is the lithiation of the C-8 position of hydroxy protected purine nucleoside with lithium diisopropylamide[11] or n-butyl lithium[12] and reaction with suitable electrophiles. Palladium catalysed coupling of alkynes[13] with 8- bromo purine nucleosides has also been reported. However there exists less precedence in the literature for C-8 functionalization of 2'-deoxy[10b,14] and 2',3'-dideoxy purine nucleosides.

In connection with our interest in functionalized 2'-deoxy and 2',3'-dideoxynucleosides we required C-8 allyl and vinyl analogues as substrates for further transformation. In this communication we report the allylation and vinylation of the C-8 position of t-butyldimethylsilyloxy derivatives of adenosine, 2'-deoxyadenosine and 2',3'-dideoxyadenosine by subjecting the 8-iodo derivatives to Pd catalysed cross coupling with allyl and vinyltributyltin[15](Table 1).

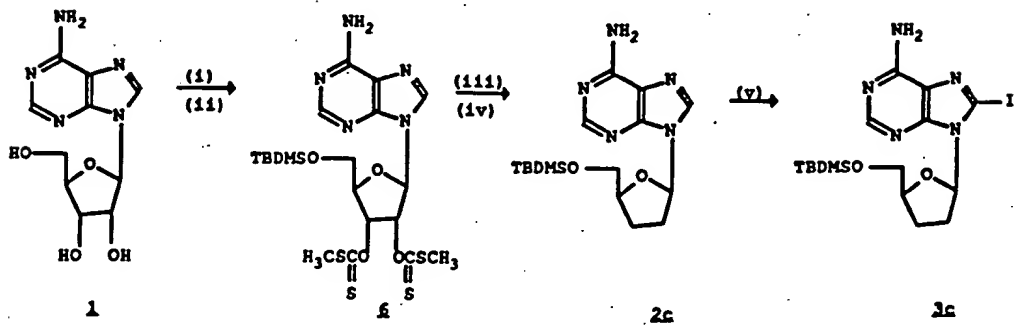
Iodination of the C-8 position using a procedure similar to Miyasaka's for 8-iodo cordycepin[16a], namely lithiation of the C-8 position of hydroxy protected (with TBDMSCl) nucleoside with LDA at -78°c in THF and quenching with iodine, yielded 3a-c (Scheme 1). The iodination proceeded with moderate to satisfactory yields[16b] [3a-(72%), 3b-(80%), 3c-(65%)]. Since protection of the OH groups with TBDMSCl made the nucleosides less polar and hence easier to handle and purify, the protecting groups were retained for the Pd catalysed reactions.

APPLICANT'S EXHIBIT C



i) LDA, -78 °c, THF, I_2 (ii) vinyltributyltin, $Pd(PPh_3)_4$, DMF, r.t. to 95 °c
(iii) allyltributyltin, $Pd(PPh_3)_4$, HMPA, r.t. to 145 °c

Scheme 1



i) TBDMSCl, imidazole, DMF (ii) NaOH, CS_2, CH_3I, DMSO (iii) Bu_3SnH, AIBN, toluene, refl.
(iv) H_2, Pd/C, CH_3OH (v) LDA, -78 °c, THF, I_2

Scheme 2

Heating 8-iodo adenosine analogues (3a-c) from r.t. to 90-95°c. with vinyltributyltin and 5 mol% $Pd(PPh_3)_4$ in DMF gave the C-8 vinyl nucleosides (4a-c) in high yields after chromatography[17]. Under the same conditions allylation did not take place satisfactorily, yielding a mixture of the desired C-8 allyl nucleoside derivative and C-8 deiodinated nucleoside derivative .This was not totally unexpected because it has been reported that aryl iodides are poor substrates for Pd catalysed allylation with allyltributyltin[18]. Success was achieved using HMPA as the solvent and increasing the temperature to 145°c. Under these conditions the reaction proceeded cleanly, yielding very little of the deiodinated starting material.

Table-1

Synthesis[17] of 8-allyl and vinyl t-butyldimethylsilyloxy derivatives of adenosine

Starting Material[b]	Product[b]	Yield %[a]	M.P.(°c)
3a	4a	92	179-180
3b	4b	89	112-114
3c	4c	90	148-150
3a	5a	81	138-140
3b	5b	89	132-133
3c	5c	75	84-85

a) Isolated yield after chromatography
b) Characterized by IR, ^1H and ^{13}C NMR and mass spectra.

5'-OTBDMS 2',3'-dideoxyadenosine (2c)was prepared following the procedure by Chu et al.[19](Scheme 2).These workers were unable to reduce the 5'-OTBDMS 2',3'-didehydro-2',3'-dideoxy adenosine to the corresponding 5'-OTBDMS 2',3'-dideoxy derivative directly with H_2/ Pd without prior deprotection of the 5'-OTBDMS group[20]. We found that this could be done directly or after pretreatment with Raney Ni[21].Iodination and coupling of the dideoxy derivative proceeded satisfactorily as well.

In summary we have synthesized C-8 allyl and vinyl derivatives of 2',3',5'-tri OTBDMS adenosine, 3',5'-di OTBDMS 2'-deoxyadenosine and 5'-OTBDMS 2',3'-dideoxyadenosine. This approach should be general for other purine nucleosides as well.

We wish to thank the National Science Foundation for the support of this work under grant CHE 8605980.

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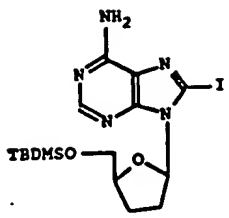
—CH ═ CH_2

4a-c

-CH=CH_2

F, r.t. to 95 °c



3c

H, AIBN, toluene, refl.

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17) In a typical vinylation reaction, to a stirred mixture of 8-iodo t-butyldimethylsilyloxy nucleoside (1eq.) and $Pd(PPh_3)_4$ (5 mol%) in DMF (under Ar), vinyltributyltin (5eq.) was added. The mixture was heated from r.t. to 90-95°c for 30-45 min. TLC showed near quantitative conversion. Workup was done by adding aq. sat. NH_4Cl, extracting with EtOAc, drying with anhyd. Na_2SO_4 and evaporating to dryness in vacuo. The crude mixture thus obtained was flash chromatographed on silicagel to yield the pure product, which crystallized either directly or upon cooling to 0°c in hexanes. For allylation a similar procedure and workup was used, except for using HMPA as the solvent and heating from r.t. to 145°c for 30-45 min. TLC again showed high conversion. Though the products 5a and 5b readily crystallized in hexanes at 0°c, crystallization of 5c was not completely satisfactory, tending to remain as a semi-solid or as an oil .
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(Received in USA 17 July 1990)

Tetrahedron Letters
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Metal-Containing Oligonucleotides: Solid-Phase Synthesis and Luminescence Properties

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La Jolla, California 92093-0358

Received November 24, 1997

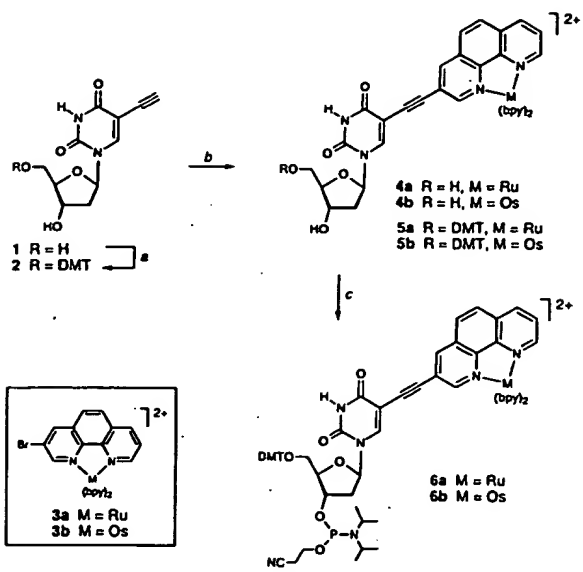
The incorporation of photo- and redox-active transition metal ions into oligonucleotides is a key design target for the study of energy and electron-transfer processes through DNA,[1] as well as the development of DNA hybridization probes and sensors.[2] Metal-containing oligonucleotides have been predominantly constructed via two major pathways: (a) the synthesis of a chelator-containing oligonucleotide followed by metal complexation[3] and (b) the synthesis of an end-functionalized oligonucleotide to which a metal complex can be conjugated.[1,4] These approaches are restricted primarily to modifications at the oligonucleotides' termini and/or require the exposure of oligonucleotides to reactive metal precursors.[2-5] A direct method for the site-specific incorporation of metal complexes during solid-phase oligonucleotide synthesis has never been reported.

We now disclose a general methodology for the incorporation of polypyridine metal complexes into oligonucleotides using automated DNA synthesizers. We report the synthesis of novel Ru^{II}- and Os^{II}-containing nucleosides and their phosphoramidite derivatives. These building blocks are sequence-specifically incorporated into oligonucleotides in high yields using standard solid-phase phosphoramidite chemistry. The uniquely modified oligonucleotides form stable DNA duplexes and are useful probes for the study of energy-transfer processes in nucleic acids.

We have previously reported that functionalized tris-chelate complexes are excellent substrates for the powerful palladium-mediated cross-coupling methodologies.[6] This approach provides a convenient entry into metal-containing nucleosides and is key to the successful preparation of the modified nucleosides and their phosphoramidites. Thus, palladium-catalyzed cross-coupling reactions between 5-ethynyldeoxyuridine[7] (**1**) and $[(bpy)_2Ru(3\text{-bromo-}1,10\text{-phenanthroline})]^{2+}(PF_6^-)_2$ (**3a**) or $[(bpy)_2Os(3\text{-bromo-}1,10\text{-phenanthroline})]^{2+}(PF_6^-)_2$ (bpy = bipyridyl) (**3b**) afford nucleosides **4a** and **4b**, respectively (Scheme 1).[8,9] The mild conditions of this reaction allow us to apply it for the modification of 4,4′-dimethoxytrityl-protected nucleosides. Thus, **1** is first treated with 4,4′-dimethoxytrityl (DMT) chloride in the presence of 4-(dimethylamino)pyridine (DMAP) to provide the DMT-protected nucleoside **2**, which is then cross-coupled to **3a** or **3b** to afford the protected metal-containing nucleosides **5a** and **5b**, respectively (Scheme 1). Phosphitylation of the protected nucleosides **5a** and **5b** using (2-cyanoethoxy)bis(diisopropylamino)-phosphine in the presence of (1*H*)-tetrazole provides the corresponding metal-modified phosphoramidites **6a** and **6b**.[10]

Target 20-mer oligonucleotides incorporating one or two metal-modified 2′-deoxyuridine bases at various positions were synthesized on a 0.2 μmol scale using an automated DNA synthesizer (Figure 1). When coupling times for the phosphoramidites **6a** and **6b** in 0.5 M (1*H*)-tetrazole were extended to 5 min, reaction efficiencies were greater than 90%.[11] Removal of the finished 20-mers from the solid support using concentrated ammonium hydroxide was followed by incubation at 55 °C for 8 h to afford

Scheme 1. Synthesis of Phosphoramidites 6a and 6b[a,b]



[a] Reagents: (a) 4,4′-dimethoxytrityl chloride (DMT-Cl), DMAP, pyridine, Et_3N, 92% yield; (b) **3a** or **3b**, $(Ph_3P)_2PdCl_2$, CuI, DMF, Et_3N, sonication, 84% yield; (c) $(iPr_2N)_2POCH_2CH_2CN$, (1*H*)-tetrazole, CH_3CN; 70−85% yield.. [b] All metal-modified nucleosides were isolated as their PF_6^- salts.[8]

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(11) To control the amount of reagents and reaction time, the coupling of the modified bases was performed manually (ref 8). The decreased coupling efficiencies relative to standard phosphoramidites are likely due to the steric bulk of the appended metal complex and have been reported with other bulky phosphoramidites. See: Kobertz, W. R.; Essigmann, J. M. *J. Am. Chem. Soc.* **1997**, *119*, 5960−5961.

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APPLICANT'S EXHIBIT D

7 5' TCG GCG CGA ATT CGC GTG CC 3'

8 5' TCG GCG CGA A[Ru]UT CGC GTG CC 3'

9 5' TCG GCG CGA A[Os]UT CGC GTG CC 3'

10 5' [Ru]UCG GCG CGA ATT CGC GTG CC 3'

11 5' TCG GCG CGA A[Os]UT CGC G[Ru]UG CC 3'

12 3' AGC CGC GCT TAA GCG CAC GG 5'

13 3' AGC CGC GC[Os]U TAA GCG CAC GG 5'

14 3' AGC CGC GCT TTA GCG CAC GG 5'

Figure 1. Sequences of oligonucleotides synthesized. The Ru^{II}- and Os^{II}-containing deoxyuridine nucleosides (4a and 4b, respectively) are shown in bold.

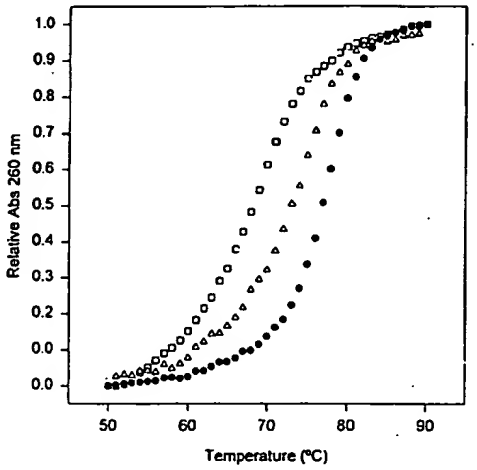

Figure 2. Thermal denaturation curves for control duplex **7·12** (●), Os^{II}-containing duplex **9·12** (△), and a single-mismatch duplex **7·14** (□) determined in 0.01 M sodium phosphate buffer pH 7, 0.1 M NaCl.[8]

the deprotected oligomers **8**−**11** and **13** that were purified by gel electrophoresis.[8] Analytical denaturing polyacrylamide gel electrophoresis confirmed the purity of the modified oligonucleotides, and enzymatic digestion followed by HPLC analysis verified the presence of the intact metal-containing nucleosides.[8]

The presence of the a metal-containing nucleoside has a relatively small effect on duplex stability as determined by thermal denaturation curves (Figure 2). The melting temperature (T_m) of the unmodified duplex derived from oligonucleotide **7** and its complementary sequence **12** is 78 °C. When the metal-containing nucleoside is located at the 5'-end, as in duplex **10·12**, the T_m is essentially the same. Duplexes **8·12** and **9·12**, in which the metal-containing nucleoside is in the middle of the duplex, are slightly less stable with a T_m at 75 °C. Yet, this destabilization is far smaller than the effect of a single mismatch on duplex stability as demonstrated for duplex **7·14** containing a T−T "pair" at the same position (T_m = 69 °C, Figure 2).

Steady-state emission profiles of iso-absorptive oligonucleotide solutions in degassed phosphate buffer are shown in Figure 3. The Ru^{II}-containing duplex **8·12** shows a typical metal-centered emission at 630 nm upon excitation of the visible metal-to-ligand charge-transfer (MLCT) band at 456 nm. Upon hybridization of the Ru^{II}-containing oligonucleotide **8** to a complementary Os^{II}-containing oligonucleotide **13**, a substantial drop in the emission intensity (ca. 70−85%) is observed. This suggests an "intra-duplex" quenching of the excited Ru^{II} center by the proximal Os^{II}

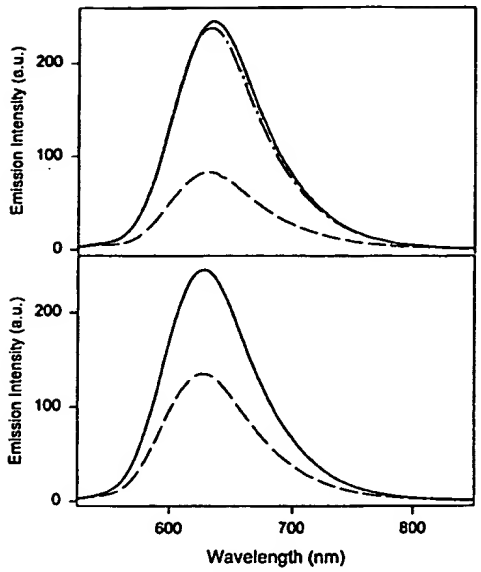

Figure 3. Steady-state emission spectra of modified oligonucleotides in degassed 0.01 M sodium phosphate buffer pH 7.0, 0.1 M NaCl. Top: duplex **8·12** (solid line), a 1:1 mixture of **8** and **9** (-·-), and a duplex containing proximal Ru and Os **8·13** (dashed line). Bottom: duplex **10·12** (solid line) and duplex **10·13** (dashed line).

center.[9-12] Intermolecular quenching can be excluded since a 1:1 mixture of the noncomplementary oligonucleotides **8** and **9** shows essentially the same emission intensity as duplex **8·12** (Figure 3a, top). This behavior is distance-dependent as demonstrated by comparing duplex **8·13** to duplex **10·13**. In this case, where the Os^{II} center is more remote, only 40% quenching of the Ru^{II}-based emission is observed (Figure 3b, bottom). To the best of our knowledge, this is the first example of energy-transfer processes in DNA oligonucleotides that are sequence-specifically modified with polypyridine metal complexes.

The data presented here establish a novel and powerful approach for the site-specific incorporation of polypyridine−metal complexes into synthetic oligonucleotides using automated phosphoramidite chemistry. The versatile phosphoramidite synthesis and the compatibility with existing automated DNA synthesizers provides enormous flexibility for rapid construction of oligonucleotide−metal conjugates. The presence of photo- and redox-active metal centers in these oligonucleotides makes them extremely important probes for the study of photophysical processes in nucleic acids.

Acknowledgment. We thank Clinical Micro Sensors, Inc., and the UC Biotechnology STAR Project for generous support of this research and Professor David R. Kearns for the use of his temperature-controlled UV spectrophotometer.

Supporting Information Available: Synthetic procedures and analytical data for all new derivatives as well as procedures and data for oligonucleotide synthesis, purification, digestion, melting, and fluorescence studies (13 pages). See any current masthead page for ordering information and Web access instructions.

JA9739998

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PATENT
Attorney Docket No.: A-63463-1/RFT/RMS/RMK

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

TOR et al.

Serial No.: 08/648,270

Filed: May 15, 1996

For: SUBSTITUTED PHENANTHROLINES

Examiner: L. E. Crane

Group: 1600

Art Unit: 1623

CERTIFICATE OF MAILING

I hereby certify that this correspondence, including listed enclosures, is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, DC 20231 on Mar 23, 2001

Signed: Suzan Lindstrom

DECLARATION UNDER 37 C.F.R. § 1.132 and M.P.E.P. § 716.09

Assistant Commissioner of Patents
Washington, DC 20231

Sir:

I, Thomas J. Meade, do hereby declare as follows:

1. I received a Ph.D. degree in Inorganic Chemistry in 1985 from The Ohio State University. I have been a Senior Research Faculty, Division of Biology and the Beckman Institute, California Institute of Technology for the last 5 years.

2. Attached to this Declaration as Exhibit A is a copy of my curriculum vitae and a list of publications.

3. I am on the Scientific Advisory Board and a consultant for Clinic Micro Sensors, the exclusive licensee of this patent application.

4. I have read and I understand the above-identified patent application, the Preliminary Amendment submitted in response to the Office Action dated July 5, 2000, the exhibits attached to the Preliminary Amendment, and the Office Action mailed January 26, 2001.

5. Based on my understanding, the present invention describes methods of making metal ion complexes comprising 1,10 phenanthroline covalently attached to nucleic acids via acetylene linkages at the 3 and/or 8 positions of the 1.10 phenanthroline moiety. The synthesis of these compounds requires: (1) 1,10 phenanthroline derivatives functionalized at the 3- and/or 8- position; (2) addition of metal ions; (3) a method for covalently attaching nucleic acids to functionalized derivatives of 1,10 phenanthroline; and, (4) the optional incorporation of metal ion complexes comprising 1,10 phenanthroline covalently attached to nucleic acids into oligonucleotides.

6. The Examiner's main point appears to be that the specification does not describe in sufficient detail the method of making transition metal complexes comprising 1,10 phenanthroline covalently attached to nucleic acids via acetylene linkages at the 3 and/or 8 positions. I disagree for the following reasons.

7. First, functionalized derivatives of 1,10 phenanthroline can be made via direct halogenation of commercially available 1,10 phenanthroline monohydrochloride monohydrate. In the specification, one such procedure is described in Example 1. In Example 1, commercially available 1,10 phenanthroline monohydrocholoride monohydrate is treated with bromine to yield two products: 3-bromo-1,10 phenanthroline and 3,8-dibromo-1,10 phenanthroline. Standard purification techniques, such as column chromatography, can be used to separate and purify the two products. In my opinion, the bromination procedure used to make functionalized derivatives of 1,10 phenanthroline is

sufficiently described to allow one of skill in the art to make these compounds without undue experimentation.

8. Second, synthesis of some of the compounds described in the invention requires the addition of metal ions. A typical reaction for adding the transition metal ruthenium to 3,8-dibromo-1,10 phenanthroline is described at page 27, lines 4-13 of the specification. It is my opinion that the procedure described on page 27 may be adapted for the addition of other transition metal ions including copper, cobalt, iron, rhodium, osmium, rhenium, etc. Thus, it is my belief that a person of skill in the art using the procedure outlined on page 27 could readily synthesize derivatives of 1,10 phenanthroline complexed to a number of different metal ions.

9. Third, methods for attaching nucleic acids to the 3-bromo- and 3,8-dibromo-1,10 phenanthroline derivatives are required to form the compounds of the invention. Palladium catalyzed cross coupling reactions are one way to covalently attach nucleic acids to functionalized derivatives of 1,10 phenanthroline. Methods using palladium catalyzed cross coupling reactions to attach nucleic acids to a wide variety of compounds are well known in the art. The references of Robins and Barr, (1983) *J. Org. Chem.*, 48:1854-1862 and Moriarty et al., (1990) *Tetrahedron Letters*, 41:5877-5880, referred to in the preliminary amendment, are examples of methods using palladium catalyzed cross coupling reactions to couple nucleosides to terminal alkynes.

10. It is my opinion, that palladium-mediated cross coupling reaction can be applied to couple nucleosides to aromatic acetylenes derivatives of 1,10 phenanthroline. Sufficient guidance for using the palladium catalyzed cross coupling reaction to couple nucleosides to aromatic acetylene derivatives of 1, 10 phenanthroline is provided in the specification in Scheme II, page 18 of the specification; Scheme IV, page 20 of the

specification; Scheme V, page 20 of the specification and Example 3. Thus, it is my belief that a person of skill in the art using the procedures outlined in the specification could readily synthesize derivatives of 1,10 phenanthroline attached to nucleosides.

11. Finally, methods for synthesizing oligonucleotides are well known in the art. For instance, solid-phase phosphoramidite chemistry may be used to synthesis the metal containing compounds described in the specification. The paper by Hurley and Tor, (1998) J. Am. Chem. Soc., referred to in the Preliminary Amendment, is an example of how the compounds of the present invention may be incorporated into oligonucleotides using solid-phase phosphoramidite chemistry. Furthermore, my lab has recently published a paper where we also incorporate transition metal-containing nucleosides (attached at either the 2' or 5' position of the ribose) into a nucleic acid using solid phase and phosphoramidite chemistry. See Rack et al., J. Amer. Chem. Soc. 122(26):6287-6288 (2000), a copy of which is enclosed as Exhibit A. Thus, it is my opinion that a person of skill in the art using solid-phase phosphoramidite chemistry could make the compounds of the invention without undue experimentation.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful, false statements may jeopardize the validity/enforceability of the application or any patent issued thereon.

Date: 3-23-01

Thomas J. Meade, Ph.D.

1014849.RMK

Communications to the Editor

Spectroscopy and Electrochemistry of Ruthenium-Modified Nucleic Acids: Design of a Novel Metal-Binding Nucleoside

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Received November 10, 1999

Electron transfer (ET) reactions through DNA[1] have been the subject of numerous investigations due to the implications for light-induced DNA damage[2] and the quest for understanding long-range ET events in biological molecules.[3] An important objective in this area continues to be the facile and site-specific incorporation of metal complexes into DNA. While recent work has focused on nucleobasic and nonnucleosidic sites for the attachment of high-potential complexes,[4] our efforts have concentrated on the ribose ring (to minimize structural perturbations) as the incorporation site for *both* high- and low-potential metal complexes.[5]

To this end, we have designed a novel chelating nucleoside (**1**) that enables the preparation of a series of metallonucleosides. We report the synthesis and spectral characteristics of a unique electron donor–acceptor pair prepared from **1** consisting of low- (**2**) and high-potential (**3**) metallonucleosides. Further, we describe the first example of a metal-containing oligonucleotide (**4**) prepared by solid-phase methods starting with the metal complex derivatized directly to a solid support.

The metal-binding nucleoside **1**, 5′-O-(4,4′-dimethoxytrityl)-2′-iminomethylpyridyl-2′-deoxyuridine, was readily prepared in situ by condensation of 5′-DMT protected 2′-amino-2′-deoxyuridine[6] with 2-pyridinecarboxaldehyde (Figure 1).[7] The ruthenated nucleosides **2** and **3** were prepared by subsequent addition of $Ru(acac)_2(CH_3CN)_2$[8] (acac = acetylacetonate) and $Ru(bpy)_2Cl_2$ (bpy = 2,2′-bipyridine) to **1**; they represent the range of metal complexes that can be inserted at the 2′-chelate site.

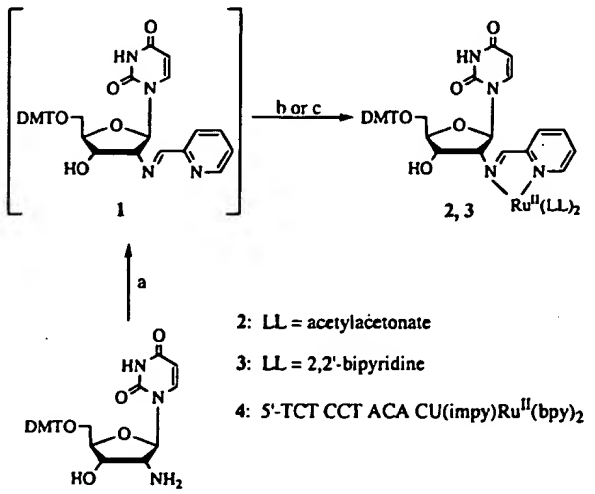

Figure 1. Synthesis and structures of 2′-ruthenated nucleosides and oligonucleotide **4**: (a) 2-pyridinecarboxaldehyde, EtOH, 2 h; (b) $Ru(acac)_2(CH_3CN)_2$, EtOH, 1 h, 79% yield; and (c) $Ru(bpy)_2Cl_2$, EtOH, 4 h, 19% yield.

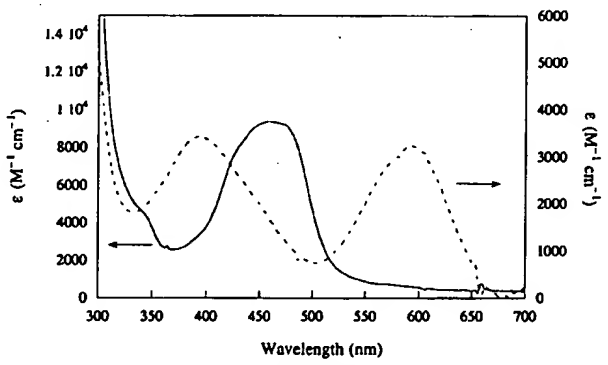

Figure 2. Absorption spectra of $[Ru(acac)_2(1)]$ (**2**) (dashed line) and $[Ru(bpy)_2(1)]^{2+}$ (**3**) (solid line) in ethanol and methanol, respectively.

Metallonucleoside **3** was selected for derivatization to the solid support due to its stability in both the mildly acidic and strongly basic solutions that are routinely encountered during automated DNA synthesis. Treatment of **3** with succinic anhydride produced the hemisuccinate form that was isolated by flash chromatography in 54% yield.[9] Derivatization of the solid support with succinated **3** enabled the preparation of oligonucleotide **4** with yields

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comparable to those observed in automated DNA synthesis. The duplex formed with **4** and its complementary sequence strand exhibited a single, cooperative melting transition with T_m = 50 °C (50 mM NaP_i, 0.5 M NaCl, pH 7.0; 2 °C higher than T_m of the corresponding unmodified duplex).

The spectral characteristics of metallonucleosides **2** and **3** are shown in Figure 2. The absorption spectrum of **2** reveals maxima at 396 (ϵ = 3600 M^{-1} cm^{-1}) and 592 nm (ϵ = 3400 M^{-1} cm^{-1}) in EtOH, which shift slightly in CH_2Cl_2 (392 nm, 3600 M^{-1} cm^{-1}; 602 nm, 3700 M^{-1} cm^{-1}). The reduction potentials of **2** and the model complex, $Ru(acac)_2(impy)$ (impy = iminomethylpyridine), are 92 and 33 mV vs Ag/AgCl, respectively. Apparently, the proximity of the nucleoside to the metal center causes the reduction potential to shift to more positive values.

The electronic spectra of **3** and **4** display a broad absorption band at 480 nm (ϵ = 9100 M^{-1} cm^{-1}), consistent with that previously reported by Keene and Meyer for $Ru(bpy)_2(impy)$.[10] This band, which is red-shifted from λ_{max} = 452 nm (ϵ = 14600 M^{-1} cm^{-1}) for $[Ru(bpy)_3]^{2+}$, reveals the effect of substituting iminomethylpyridine for bipyridine. Electrochemical measurements on **3** (in dichloromethane) and **4** (in water) give Ru(III/II) reduction potentials of 1.4 and 1.1 V vs Ag/AgCl, respectively.

Irradition of **3** and **4** at 480 nm yields identical lifetimes (λ_{max}(em) = 740 nm; τ = 42 ns; $\Phi = 1.1 \times 10^{-4}$), which are shorter than that observed for $[Ru(bpy)_3]^{2+}$ (λ_{max}(em) = 625 nm; τ = 620 ns; Φ = 0.042).[11] Indeed, the differences in lifetime and quantum yield correspond to an increase in the nonradiative rate constant (k_{nr}) from 1.54×10^6 s^{-1} for $[Ru(bpy)_3]^{2+}$ to 2.38×10^7 s^{-1} for **4**. The radiative rate constant (k_r) for **4** is on the order of 10^3 s^{-1}, suggesting that the emissive state is similar to other $Ru(bpy)_3^{2+}$-type chromophores. In comparison to $[Ru(bpy)_3]^{2+}$, the iminomethylpyridine ligand in $[Ru(bpy)_2(impy)]^{2+}$ is responsible for the red-shifted emission and the increase in k_{nr}.

We utilized resonance Raman (rR) spectroscopy to investigate the nature of the visible transitions of these complexes. The rR spectrum of **4** (identical to **3**; 441.6 nm excitation) is similar to that of other ruthenium polypyridyl compounds (Figure 3).[12] The peaks at 1023, 1173, 1276, 1316, 1488, 1552, and 1604 cm^{-1} correspond well to those observed in the rR spectrum of $[Ru(bpy)_3]^{2+}$.[13] Additional peaks (1242, 1471 cm^{-1}) represent excited-state distortions that are not present in $[Ru(bpy)_3]^{2+}$, and can be attributed to the impy ligand.

Despite the weak absorption of **2** at the available excitation frequencies (441.6 and 514.5 nm), rR spectroscopy provided valuable data. The rR spectrum (441.6 nm excitation) of **2** reveals

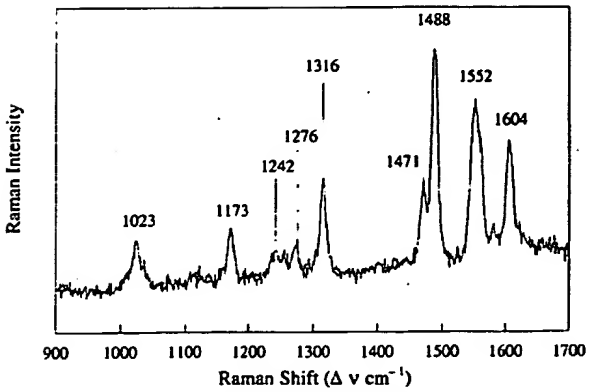

Figure 3. Resonance Raman spectrum of oligonucleotide **4** in unbuffered aqueous solution.

characteristic acac vibrations at 1528 (ν(CO)) and 1234 cm^{-1} (ν(C−C)).[14,15] Spectral features typically seen for polypyridyl complexes are absent, confirming the Ru dπ → acac π^* nature of the transition.[16] The spectrum obtained with 514.5 nm excitation yields vibrations similar to those observed for complex **4** (1249, 1288, 1501, 1530, 1551, and 1597 cm^{-1}). As a result, the low-energy band at 592 nm is assigned as a Ru dπ → impy π^* transition.

The synthesis of both the low-potential complex **2** and the metal-modified solid support represents a significant advance in the development of metal-containing oligonucleotides. The synthetic versatility of **1** is demonstrated by the preparation of **2** and **3**. Further, the distinct absorption and electrochemical features of these complexes are well-suited for ground-state ET studies involving DNA. Future work will examine these characteristics and focus on additional means of incorporating similar oxidants.

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Supporting Information Available: Instrumental details and experimental procedures for complexes **1**−**4** and $Ru(acac)_2(impy)$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Education

Postdoctoral Research Fellow. California Institute of Technology, 1987-89. Advisor: Professor Harry B. Gray.

National Institutes of Health Postdoctoral Fellow. Harvard Medical School and the Massachusetts General Hospital, 1985-1987. Advisor: Professor Thomas J. Brady.

Ph.D. in Inorganic Chemistry, The Ohio State University; Columbus, Ohio, August, 1985. Dissertation Advisor: Professor Daryle H. Busch.

M.S. in Chemistry (Division of Biochemistry), The Ohio State University, Columbus Ohio, 1982. Thesis Advisor: Professor Perry A. Frey.

B.S. with Honors in Chemistry, Arizona State University; Tempe Arizona, May, 1980. Honors Thesis Advisor: Professor Therald Moellar.

Professional Experience

Senior Research Faculty, Division of Biology and the Beckman Institute, California Institute of Technology, (1996-present).

Director: Program for Bioinorganic Drug Design and Discovery, Beckman Institute, California Institute of Technology, (1993-present).

Research Faculty: Division of Biology, California Institute of Technology, (1991-96).

Research Interests

Chemistry of Life Sciences, bioinorganic chemistry and biological imaging with particular emphasis on:

INORGANIC CHEMISTRY- Design, synthesis and physical properties of coordination complexes, including systems incorporating novel functionality for magnetic resonance and fluorescence imaging of biological systems.

ELECTRON TRANSFER MECHANISMS- Investigate long-range electronic coupling through stacked, π-unsaturated systems, conducting biopolymers and the development of biosensors

BIOLOGICAL MICROSCOPY- Design and synthesis of spectroscopic and magnetic probes for *in vivo* microscopic imaging of nerve patterning, regulation of cell lineage, gene expression and DNA transfection.

METAL COMPLEXES AS ENZYME INHIBITORS- Investigate the interaction of small-molecule transition metal complexes as enzyme inhibitors for the development of therapeutic antitumor and antiviral drugs.

Honors and Awards

National Academy of Engineering Lecturer, Cleveland, Ohio, 2000.
Pendergast Lecturer, University of Pennsylvania, 1999.
American College of Neuropharmacology Lecturer, 1999.
Grubstakes Award, Direct Detection of Gene Expression via Magnetic Resonance Imaging; Caltech, 1996, 1998.
Watson Lecturer, Caltech, 1997.
NIH Postdoctoral Research Fellow, 1986.
National Research Service Award, Harvard Medical School, 1985.
Ohio State University Teaching Award (student selected), 1981.
B.S. with Honors Thesis, 1980.

Professional Service

Editorial Advisory Board: Inorganic Chemistry; 1999-
Program Workshop Chair, CTEP drug development program, National Cancer Institute; 1999-2000.
Editorial Advisory Board: Bioconjugate Chemistry; 1999-.
Ad hoc member; NIH Metallobiochemistry Study Section; 1999-.
Chairmen: Imaging in 2020; National Cancer Institute; 1999
Founder and Scientific Advisory Board Member, Metaprobe LLC Pasadena, CA. 1998-present.
Chairmen: After The Genome IV Conference; 1998.
Guest Editor: Coordination Chemistry Review, 1998-99.
Cofounder and Scientific Advisory Board Member, Clinical Micro Sensors Inc., Pasadena, CA. 1995-present.
11 named and 73 Invited lectures; 1992-2000.

Teaching

Supervised undergraduate and graduate laboratories and led recitation sections (O.S.U. 1980-81). Ohio State University Teaching Award (student selected) 1981.

Codirector and Founder; "Seeing is Believing: A Classroom Tour of the Sciences." Pasadena Unified School District and local private schools, 1994-present.

Affiliations

American Chemical Society, American Association for the Advancement of Science, International Society for Magnetic Resonance in

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Medicine, Sigma Chi Honorary Research Society, Alpha Chi Sigma, New York Academy of Sciences, Society for Molecular Imaging.

Trainee History

Supervised 16 undergraduates, 9 graduate students and 16 post-doctoral fellows, 1992-present.

Publications

Meade, T.J., Iyenger, P.A., Frey, P.A., "Synthesis and Rearrangements of Alkyl Phosphorothioates", Journal of Organic Chemistry, **1985**, 50, 936.

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